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Begin the article with a short summary.

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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1980.

Phase II Trial of Piperazinedione in Hodgkin's Disease, Non-Hodgkin's Lymphoma, and Multiple Myeloma: A Southwest Oncology Group Study [1,2,3,4]

Stephen E. Jones,* William G. Tucker, Arthur Haut, Bill L. Tranum, Clarence Vaughn, Ellen M. Chase, and Brian G. M. Durie [5]

SUMMARY

Piperazinedione given iv once every 3–4 weeks at a starting dose of 9–12 mg/m^2 (4.5–12 mg/m^2 for patients with multiple myeloma) was evaluated in a Southwest Oncology Group phase II study for patients with far-advanced refractory lymphoma or multiple myeloma. Among 36 patients fully evaluable for tumor response (adequate trial), partial responses were observed in five (71%) of seven patients with Hodgkin's disease, in three (19%) of 16 patients with non-Hodgkin's lymphoma, and in none of 13 patients with multiple myeloma. Response was observed by the time of the second (five patients) or third (three patients) course. The median duration of response was 3.7 months (range, 1–17+ months). The dose-limiting toxic effects were hematologic, with 18 (50%) of 36 patients evaluable for toxicity experiencing severe leukopenia (wbc count $<$ 2000/mm^3) and 22 (61%) experiencing severe thrombocytopenia (platelet count $<$ 50,000/mm^3). Twenty patients had a decrease from their pretreatment hemoglobin level of $\geq$ 2 g/100 ml. Hematologic toxic effects were often unpredictable and in several patients quite prolonged. This study indicates that piperazinedione has definite antitumor activity in patients with Hodgkin's disease and further trials in this disease using the drug at a reduced dose in combination with other effective drugs appear warranted.

[Cancer Treat Rep 61:1617–1621, 1977]

Piperazinedione is an antitumor antibiotic which was isolated from the fermentation broth of *Streptomyces griseoluteus* by Gitterman et al in 1970 (1). In preclinical studies, the drug has been shown to inhibit the growth of transplantable human tumors in the chick embryo (1), as well as murine and human tumor cells in culture (2,3), and to have activity against a wide spectrum of transplantable rodent tumors (including P388 lymphocytic leukemia, AKR leukemia, Lewis lung carcinoma, Walker

[1]Received June 27, 1977; revised Aug 5, 1977; accepted Aug 18, 1977.

[2]Supported by Public Health Service grants CA-03195, CA-13612, CA-04915, CA-03389, CA-04920, CA-03392, CA-16957, CA-04919, CA-16943, and CA-12014 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Piperazinedione (NSC-135758) was supplied by the Division of Cancer Treatment, NCI, Bethesda, Md.

[4]Presented in part at the annual meeting of the American Society of Clinical Oncology, May 1976, Toronto, Ontario (Abstr No. 179, Proc Am Assoc Cancer Res and ASCO 17:45, 1976).

[5]This study was conducted by the Southwest Oncology Group (SWOG). The following institutions and investigators participated in this study: University of Arizona College of Medicine, Tucson (*S. Jones, B. Durie, S. Salmon,* and *E. Chase*); University of Arkansas Medical Center, Little Rock (*A. Haut* and *B. Tranum*); Baylor College of Medicine, Houston, Tex (*M. Lane*); Borgess Medical Center, Kalamazoo, Mich (*R. Lord* and *W. Tucker*); Brooke General Hospital, Brooke Army Medical Center, Fort Sam Houston, Tex (*J. McCracken*); Cleveland Clinic, Ohio (*J. Hewlett* and *J. Weick*); Henry Ford Hospital, Detroit, Mich (*R. Monto* and *E. Van Slyck*); Ohio State University Hospitals, Columbus (*H. Wilson*); University of Oklahoma Health Sciences Center, Oklahoma Medical Research Foundation, Oklahoma City (*R. Bottomley* and *T. Self*); Providence Hospital, Southfield, Mich (*C. Vaughn*); University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston (*R. Alexanian*); Tulane University School of Medicine, New Orleans, La (*W. Stuckey*); and Wilford Hall USAF Medical Center, Lackland Air Force Base, Tex (*C. Coltman*).

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256 carcinosarcoma, Ehrlich ascites tumor, B16 melanoma, and intracranially implanted ependymoblastoma) (4).[6] The drug is highly active against L1210 leukemia in the mouse in both daily and intermittent schedules. Of particular interest are animal studies showing the drug to be active against alkylating agent-resistant L1210 leukemia in the mouse (4–6).

Although the mechanism of action of piperazinedione is not completely understood, several effects are apparent: (*a*) it blocks cell cycle progression at the G_2 phase (although progression of cells to the G_2 phase during the period of exposure does not appear to be essential for cell killing); (*b*) it inhibits DNA synthesis; and (*c*) it acts as an alkylating agent (2,3,7). Additional studies in L1210 leukemias sensitive or resistant to alkylating agents have indicated that there are important differences in the biologic activity of piperazinedione compared with cyclophosphamide, melphalan, or BCNU (3,6).

During phase I trials conducted by Gottlieb et al (8) studying single doses given iv once every 3–4 weeks, piperazinedione was found to have clinical activity in several different tumor types including chronic myelogenous leukemia in blast cell crisis and acute myelogenous leukemia. The dose-limiting toxicity was hematologic. On the basis of these encouraging results in patients with extensive prior therapy and the animal screening data, the SWOG began a phase II trial of piperazinedione in patients with refractory Hodgkin's disease, non-Hodgkin's lymphoma, or multiple myeloma. This report describes our experience with this new agent.

PATIENTS AND METHODS

Eligibility Criteria

Patients of any age with Hodgkin's disease, non-Hodgkin's lymphoma, or multiple myeloma who were refractory to prior conventional therapy with combination chemotherapy were eligible for entry into this study. Additional eligibility criteria included:

(*a*) initial granulocyte count $\geq 2000/mm^3$;
(*b*) platelet count $> 100{,}000/mm^3$;
(*c*) no chemotherapy for 3 weeks prior to the start of piperazinedione therapy;
(*d*) no radiation therapy to measurable lesions within the preceding 3 months;
(*e*) measurable tumor or myeloma M-component (monoclonal immunoglobulin) to judge response;
(*f*) submission of original biopsy material for pathology review (for patients with lymphoma) (9). [The Lukes-Butler and Rappaport histopathologic classification systems were employed (10–12).]

Treatment Schedule

On the basis of phase I data (8), piperazinedione was administered at an initial dose of 12 mg/m^2 iv every 3–4 weeks. (This was subsequently reduced to 9 mg/m^2 for patients with impaired bone marrow reserves.) Subsequent dose modification (escalation or reduction) was based on the degree of hematologic toxicity. Eight patients with multiple myeloma received the drug at an initial dose of 4.5–8 mg/m^2 (with subsequent escalation) according to the same schedule at the M. D. Anderson Hospital prior to formal activation of the Group-wide study; these cases are included in this series.

Study Parameters

Baseline and serial examinations which were performed included physical examination, complete hemogram with complete blood cell, differential, and platelet counts; determination of serum creatinine, uric acid, electrolyte, calcium, bilirubin, alkaline phosphatase, SGOT and BUN levels; urinalysis; chest roentgenograms; and tumor measurements. For patients with multiple myeloma, creatinine clearance, quantitative serum immunoglobulin and serum and urine M-component determinations, bone marrow examination, and metastatic bone survey were also required.

Response Criteria

Patients who received an adequate trial (two courses of therapy embracing a 6-week observation period or clear progression of disease after a single injection with myelosuppression) were considered evaluable for response. For patients with lymphoma, response was defined as follows:

(*a*) *partial remission*— >50% decrease of measurable lesions for at least 1 month;
(*b*) *improvement*—some evidence of objective tumor regression, but < 50% reduction in tumor;
(*c*) *no response*—stable disease despite at least two courses of treatment;
(*d*) *progression*—> 50% increase in known lesions or the appearance of significant new lesions;
(*e*) *relapse*—appearance of new lesions or the reappearance of old lesions in patients who had previously achieved an objective response.

For patients with multiple myeloma, objective re-

[6]Clinical brochure: piperazinedione, NSC-135758. Prepared by the NCI, Bethesda, Md, June 1975.

TABLE 1.—Phase II evaluation of piperazinedione (SWOG study 7435): patient entry and evaluability data

No. of patients—	Total No. of patients	Hodgkin's disease	Non-Hodgkin's lymphoma	Multiple myeloma
Eligible	44	8	23	13
Evaluable for response	36	7	16	13
Partially evaluable (early death)*	7	1	6	—
Not evaluable (major protocol violation)	1	0	1	—

*4 patients died of tumor within 1 wk of treatment without any followup information.

sponse was assessed on the basis of regression of myeloma cell mass as calculated by the method of Durie and Salmon (13).

RESULTS

Between December 1974 and October 1976 when the study was closed, a total of 36 patients were entered into this study at starting doses of 9–12 mg/m^2 of piperazinedione. As mentioned previously, eight additional patients with multiple myeloma who received reduced initial doses (4.5–8 mg/m^2) on a pilot basis prior to the formal activation of the study were also registered and these patients are included in this report.

Patient entry and evaluability data for these 44 patients are shown in table 1. Thirty-six of the 44 eligible patients were considered fully evaluable for response. Seven patients were considered partially evaluable (early death), but had no repeat tumor measurements. One patient was inevaluable due to a major protocol violation.

All patients had advanced disease and had received extensive combination chemotherapy prior to this trial. Four patients with Hodgkin's disease and four patients with non-Hodgkin's lymphoma had also had extensive radiation therapy. The median age of all 44 eligible patients was 52.5 years (mean, 50 years; range, 19–97 years). (For patients with Hodgkin's disease, the median age was 25.5 years [mean, 29 years; range, 19–49 years].) Twenty-five of the 44 patients were men and 19 were women. Twenty-nine of the 30 partially or fully evaluable patients with lymphoma had stage IV disease and 23 of the 30 had constitutional symptoms at the time piperazinedione treatment was begun. The histologic types of lymphoma for the partially or fully evaluable lymphoma cases included mixed cellularity (two patients) or nodular sclerosis (six patients) Hodgkin's disease, and nodular lymphocytic (six patients), diffuse histiocytic (ten patients), diffuse mixed (two patients), or diffuse lymphocytic (four patients) lymphoma. Eight of the 16 fully evaluable patients with non-Hodgkin's lymphoma and three of the seven fully evaluable patients with Hodgkin's disease had proven bone marrow involvement at the start of treatment. All patients with multiple myeloma were refractory to alkylating agents and had measurable serum and/or urine M-components.

Ten (26%) of the 39 patients who lived for at least 3 weeks received one course, 13 (33%) received two courses, five (13%) received three courses, and 11 (28%) (including six of the responding patients) received more than three courses. Treatment at 21–28-day intervals with dosage escalation was possible in only ten of the 39 patients. For all other patients, either decreased doses or increased intervals between treatments (or both) were necessary to produce acceptable levels of myelosuppression. Of the 12 patients with multiple myeloma who received at least two courses, three required intervals of 110–229 days before the second course could be given. In contrast, all seven evaluable patients with Hodgkin's disease were able to receive a second course of treatment by Day 22. The other patients who received a second course (nine with multiple myeloma and ten with non-Hodgkin's lymphoma) had treatment intervals ranging up to 47 days. Only ten patients with non-Hodgkin's lymphoma received more than one course.

Response

The results of the treatment for all 36 patients evaluable for response are shown in table 2. Piperazinedione produced objective partial responses in five of seven patients with Hodgkin's disease (four with nodular sclerosis Hodgkin's disease and one with mixed cellularity Hodgkin's disease) and in three of 16 patients with non-Hodgkin's lymphoma (all with nodular lymphocytic poorly differentiated lymphoma). The predominant sites of response were the lymph nodes (five patients), bone marrow (one

TABLE 2.—Phase II evaluation of piperazinedione (SWOG study 7435): response by disease type*

Disease category	No. of fully evaluable patients (adequate trial)	No. of patients (%) who had—			
		Partial response	Improvement	No response	Increasing disease
Hodgkin's disease	7	5 (71%)	—	1	1
Non-Hodgkin's lymphoma	16	3 (19%)	1	4	8
Multiple myeloma	13	—	1	11	1

*For definitions of response, *see* text.

patient), and both (two patients). Liver and lung lesions responded in two patients as well. One additional patient with lymphoma and one with multiple myeloma manifested some improvement but less than that of a partial response. No complete responses were obtained. Responses were generally brief. For patients with Hodgkin's disease, the median duration of response was 2.5 months (1, 2, 2.5, 3, and 5 months). For patients with non-Hodgkin's lymphoma, remission durations were 3.5, 4, and 17+ months. Only one patient continues to receive treatment (at a greatly reduced dose). Response was observed by the second (five patients) or third (three patients) course. Three of the responding patients had a starting dose of 12 mg/m^2 and the other five had a starting dose of 9 mg/m^2.

Toxicity

Myelosuppression was the dose-limiting toxic effect in this study; it was often severe and unpredictable. The drug was tolerated better by patients with Hodgkin's disease (who tended to be younger) than by patients with non-Hodgkin's lymphoma or multiple myeloma in whom severe and often persistent thrombocytopenia was observed. Although nadir blood cell counts were not always available, the lowest wbc and platelet counts recorded for each patient are shown in table 3. Severe leukopenia (wbcs < 2000/mm^3) was observed in three (43%) of seven evaluable patients with Hodgkin's disease, 11 (61%) of 18 patients with non-Hodgkin's lymphoma, and four (36%) of 11 patients with myeloma who were evaluable for hematologic toxicity. Severe thrombocytopenia (platelet count < 50,000/mm^3) was observed in two (29%) of seven patients with Hodgkin's disease, 14 (77%) of 18 patients with non-Hodgkin's lymphoma, and six (54%) of 11 patients with multiple myeloma.[7] Serious hemorrhagic complications related to severe or life-threatening thrombocytopenia (observed platelet counts, 1000–49,000/mm^3) occurred in ten of the 39 patients. Three of these ten patients had multiple myeloma and four of the remaining seven patients had lymphomatous involvement of the bone marrow that was documented by bone marrow biopsy prior to treatment or was discovered at autopsy. Serious bacterial infectious episodes occurred in eight leukopenic patients. Rbc transfusions were required in 16 patients; four of these 16 patients also required platelet transfusions. A decrease in hemoglobin of ≥ 2 g/100 ml compared with the pretreatment level was observed in 20 patients. Piperazinedione was implicated as a factor in the death of four patients: one patient with multiple myeloma who had probable (but not documented) central nervous system bleeding, one patient with Hodgkin's disease who died of pneumonia and tumor, and two patients with non-Hodgkin's lymphoma who died of sepsis.

In general, patients with Hodgkin's disease who were younger and had less bone marrow involvement tolerated treatment better than patients with non-Hodgkin's lymphoma or myeloma. Nadir blood cell counts occurred on about Day 14 in patients with Hodgkin's disease followed by recovery from myelosuppression by Day 21. For the other patients, nadir blood cell counts often were not observed until 1–2 weeks later requiring delays in treatment. On occasion, even patients who appeared to be excellent candidates for treatment with piperazinedione had profound and persistent hematologic toxic effects with the first dose.

Other toxic effects noted in this study were nausea and vomiting (usually only mild to moderate). These were experienced by about one half of the patients, but were much less severe than the effects associated with most alkylating agents. No liver or renal function abnormalities were observed as a result of treatment. One patient was reported to have a mild fever.

DISCUSSION

This clinical trial of piperazinedione was initiated

[7] 1 patient with multiple myeloma and 1 patient with non-Hodgkin's lymphoma could not be evaluated for hematologic toxicity because of inadequate data.

TABLE 3.—Phase II trial of piperazinedione (SWOG study 7435): hematologic toxicity in partially and fully evaluable patients*

Hematologic parameters (lowest recorded count)	Hodgkin's disease (7 patients)	Non-Hodgkin's lymphoma (18 patients)	Multiple myeloma (11 patients)
Leukopenia (wbcs/mm³)			
> 4000	4	3	0
3000–3999	0	1	2
2000–2999	0	3	5
1000–1999	2	8	3
< 1000	1	3	1
Thrombocytopenia (platelets/mm³)			
≥ 100,000	2	1	4
75,000–99,000	0	2	1
50,000–74,999	3	1	0
25,000–49,999	0	4	4
< 25,000	2	10	2

*Followup wbc and platelet counts were not available for all partially and fully evaluable patients.

because of the activity of the agent in animal screening systems and because of its apparent antileukemic effect in man (8). In this study, the drug proved to have antitumor effects primarily in patients with Hodgkin's disease (five partial responses in seven evaluable patients). Unfortunately, none of the responses were complete and the median duration of response was short (about 4 months). Piperazinedione appears to have little activity in late-stage refractory multiple myeloma or non-Hodgkin's lymphoma. Potential antitumor activity is limited by severe, sometimes persistent, and frequently unpredictable hematologic toxicity.

Since complete cross resistance between piperazinedione and alkylating agents has not been demonstrated in animal systems and because piperazinedione does appear to have activity in Hodgkin's disease, further trials as a single agent employing starting doses of 12 mg/m² for patients with adequate bone marrow reserves and 6–9 mg/m² for those with advanced age or impaired bone marrow reserves, or in combination at reduced dosage with other effective drugs earlier in the course of Hodgkin's disease, appear warranted.

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Comparative Trial of Combination Chemotherapy in Extensive Squamous Carcinoma of the Lung: A Southwest Oncology Group Study[1,2]

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SUMMARY

The Southwest Oncology Group carried out a comparative study of two combination chemotherapy regimens in 231 patients with extensive squamous carcinoma of the lung. One regimen consisted of bleomycin, adriamycin, CCNU, vincristine, and mechlorethamine (BACON). The other involved mechlorethamine, adriamycin, and CCNU (NAC) in the same dose and schedule but with the deletion of vincristine and bleomycin. The response rate was 21% for BACON and 16% for NAC. The median survival time (MST) was 16 weeks for all patients receiving each regimen. Pretreatment performance status (PS) was significantly related to the response rates: these were 28% for BACON and 23% for NAC among fully ambulatory patients (PS, 8–10) versus 15% for BACON and 10% for NAC among those with a lesser PS ($P < 0.05$). Survival was also influenced by PS; the MST was 28 weeks for PSs 8–10 with either regimen compared to an overall MST of 11.5 weeks for patients with PSs 5–7 and 2.8 weeks for PSs 1–4. The toxicity of the two regimens was comparable with a 4% incidence of treatment-related deaths. An additional 10% of the patients were hospitalized because of life-threatening but reversible toxic effects. The only patients for whom there may be an improvement in MST over that expected from supportive care alone are those with PSs 8–10. Patients who demonstrated response or improvement, regardless of PS, had a significantly longer MST: 39.5 versus 11.2 weeks for those who did not respond ($P = 0.001$).

[Cancer Treat Rep 61:1623–1629, 1977]

The SWOG embarked on the current study in 1974, based on the experience of Livingston et al (1), whose pilot study of the BACON five-drug combination resulted in a 45% response rate in 38 patients with extensive squamous carcinoma of the lung and a median survival time (MST) of 16 weeks. Significantly superior survival was noted for responders and "stable" patients when compared to nonresponders (26 versus 9 weeks). The aims of the SWOG were (*a*) to attempt to confirm these results, and (*b*) to evaluate the efficacy of a simpler, three-drug regimen in which vincristine and bleomycin were deleted.

MATERIALS AND METHODS

The regimens employed are shown in figure 1. Appropriate reductions in the dose of the myelosuppressive agents were made if a patient developed fever and/or documented infection related to leukopenia, bleeding related to thrombocytopenia, or a granulocyte count nadir of $\leq 500/mm^3$ or platelet

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count nadir of $\leq 50{,}000/mm^3$ from the previous course of therapy. Patients were considered eligible for either treatment arm if they had squamous carcinoma of the lung as documented by the pathologist's report for each institution and met the following criteria for extensive disease, as originally defined by the Veterans' Administration Lung Cancer Study Group (VALCSG): evidence of spread of tumor beyond the hemithorax and/or prior radiation therapy with regrowth of the primary lesions. There were no exclusions from eligibility because of cardiovascular or pulmonary status, performance status (PS), or superior vena caval syndrome. Patients with disease progression after prior chemotherapy and those with renal failure were excluded. After stratification in one of three Karnofsky PS groups (8–10, 5–7, or 1–4), patients were randomized to receive either BACON or NAC by the biostatistical office of the SWOG in Houston.

The usual criteria of the SWOG were applied in the definitions of tumor response; complete response (CR) required the disappearance of all measurable disease, and partial response (PR) required a reduction by at least 50% in the sum of the areas of all measurable disease without an increase in the size of any lesion or the appearance of any new lesions. The area of a lesion was estimated by multiplying the two longest measured perpendicular diameters. All responses had to last for a minimum of 4 weeks to qualify as CRs or PRs. "Improvement" required a similar duration of objective response, but of a lesser degree than 50%. All other patients were considered nonresponders.

All patients were considered eligible who received any treatment as long as it was given according to the protocol, and all were considered partially or fully evaluable for response and survival determinations. Five were excluded because of major protocol violations. In the category "fully evaluable," the following groups are excluded: (*a*) early deaths (< 2 weeks on study), (*b*) patients lost to followup, and (*c*) patients who refused further treatment and were removed from the study for that reason. Such patients were considered partially evaluable. In the case of the three patients lost to followup, their survival was calculated as that obtained by assuming death on the last date of known followup. Survival in all patients was calculated from the date of entry in the study.

With reference to statistical considerations, response rates were compared by the chi-square test, using the standard correction for continuity. Survival comparisons were made by the Wilcoxon-Gehan test (2), and the survival curves were plotted by the Kaplan-Meier technique (3). All P values cited imply a two-sided test of significance, unless there was a priori evidence to support the hypothesis of superiority of one group over another (eg, survival in PSs 8–10 versus survival in PSs 1–4), in which case a one-sided test was employed.

RESULTS

Response

The distribution of certain prognostic factors which resulted from the randomization process is shown in table 1. At least with respect to these variables (PS, age ≥ 70 years, and presence of liver or brain metastases), patients receiving BACON versus NAC appear quite comparable. Age ≥ 70 years and the presence of liver or brain metastases were examined because of their reported significance with respect to survival in the previous study by Lanzotti et al (4). These and certain other pretreatment variables of possible prognostic significance are now being analyzed with respect to SWOG studies and will be the subject of another publication.[4]

As seen in table 2, the response rates to BACON and NAC are similar in all respects. Only among patients without prior radiation therapy is there a suggestion of superiority for BACON. The response rate appears higher for BACON-treated patients if they had not received prior radiation (30% versus 15%), while there is no difference for patients receiving NAC (15% versus 16%). However, none of these differences are significant at $P \leq 0.05$. Patients with PSs 8–10 did have higher response rates overall ($P < 0.05$).

Survival within each PS category is plotted in figure 2. The survival of patients in PSs 8–10 is superior to that of those in PSs 5–7 ($P = 0.001$) regardless of treatment, and the survival of patients in PSs 5–7 is superior to that of patients in PSs 1–4. There is no difference between BACON and NAC for PSs 8–10 or 1–4, but NAC is superior to BACON ($P = 0.05$) for PSs 5–7. The survival of patients who had had prior radiation therapy is no different from those who had had none (fig 3).

Responding and improved patients had much better survival than those who failed to respond (39.5 versus 11.2 weeks, $P = 0.001$) as shown in figure 4. The survival of all BACON responders was not superior to that of all NAC responders (MST 41.4 versus 33.8 weeks), and the median duration of response was also similar (16 and 17 weeks, respectively). If responders are analyzed within each PS category, however, fully ambulatory

[4]Heilbrun L, and Livingston R. Manuscript in preparation.

TABLE 1.—Prognostic factor distribution

Factor	No. of patients (%) BACON	NAC
PSs 8–10	54 (47)	53 (46)
Age ≥ 70 yrs	10 (9)	13 (11)
Liver metastases	22 (19)	18 (16)
Brain metastases	18 (16)	13 (11)

TABLE 2.—SWOG 7439: response to BACON vs NAC

	No. of patients (%) BACON	NAC	Total	
Evaluable	116	115	231	
Fully evaluable	98	94	192	
Response + improved, evaluable	26 (22)	20 (17)		
Response, evaluable	24 (21)	18 (16)		
Response, fully evaluable	24 (25)	18 (19)		
Response, no prior radiation	16 (30)	9 (15)		
Response, prior radiation	8 (13)	9 (16)		
Response, PSs 8–10	15 (28)	12 (23)	27 (25)	$P < 0.05$
Response, PS < 8	9 (15)	6 (10)	15 (12)	

BACON: vincristine, 0.75–1.0 mg iv in AM on Day 1, repeat once weekly × 6 doses, then stop permanently;
bleomycin, 30 units iv, 6 hrs after vincristine on Day 1, repeat once weekly × 6 doses, then stop permanently;
mechlorethamine, 8 mg/m^2 iv on Day 1, repeat every 4 wks;
adriamycin, 40 mg/m^2 iv on Day 1, repeat every 4 wks;
CCNU, 65 mg/m^2 orally on Day 1, repeat every 8 wks.

NAC: identical to BACON, but vincristine and bleomycin are deleted.

Bleomycin discontinued at a total dose of 180 units.

Adriamycin discontinued at a total dose of 450 mg/m^2.

FIGURE 1.—Drug regimens used in this study.

patients receiving BACON fared somewhat better than the same group receiving NAC (MST 52 versus 40 weeks, $P = 0.02$). There was no significant difference in the survival of responders receiving BACON versus NAC in the PS 1–7 category (MST 20 versus 23 weeks, respectively, $P = 0.582$).

Toxicity

Adequate information to evaluate toxicity was obtained in 217 patients (table 3). The overall incidence of life-threatening or fatal toxicity was 11% for BACON and 16% for NAC, almost entirely related to myelosuppression (table 3). There were six patients with documented episodes of sepsis (2.8%), three who were receiving each treatment regimen. The median lowest granulocyte count was 1100/mm^3 (range, 100/mm^3 to no change) and the median lowest platelet count was 145,000/mm^3 (range, 500/mm^3 to no change). There was no difference in severity of myelosuppression between BACON and NAC, nor any evidence of cumulative myelosuppression from subsequent courses.

A bleomycin-related pulmonary death occurred in one patient; no deaths occurred which could be related to adriamycin-induced cardiomyopathy. Only one patient in the entire study had therapy discontinued because of possible adriamycin-in-

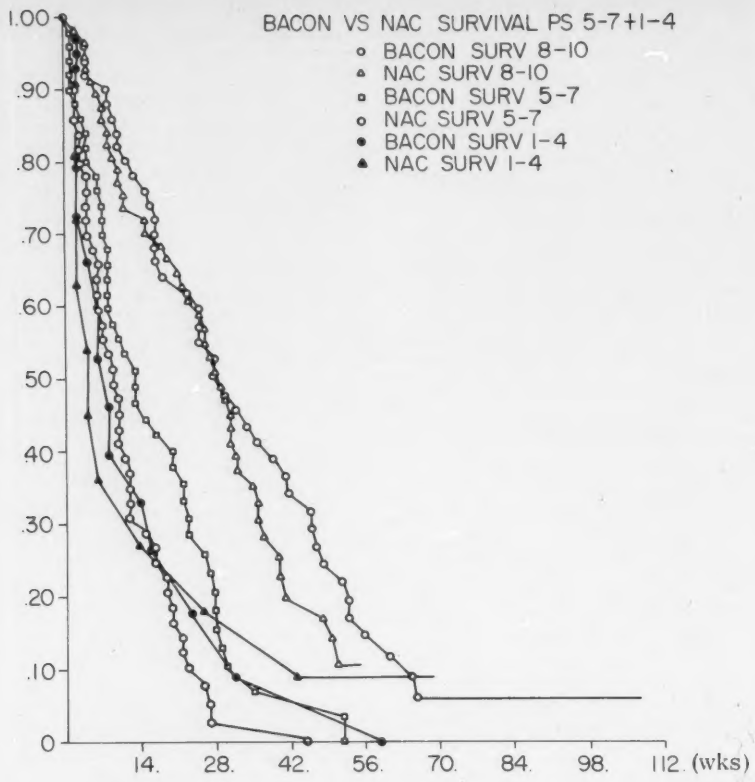

FIGURE 2.—Survival (SURV) by PS (8–10, 5–7, and 1–4) and treatment arm.

duced heart damage; he had developed paroxysmal atrial tachycardia while receiving the drug which subsequently resolved.

Toxicity rated maximally as "severe" occurred in one third of the patients and again was related primarily to myelosuppression, usually to a nadir granulocyte count of $< 1000/mm^3$ (wbc count, 1000–$2000/mm^3$). Alopecia was universal and frequently total. Nausea was observed in most patients after the monthly administration of adriamycin and mechlorethamine with or without CCNU, and the majority had vomiting associated with it. However, the qualitative severity of this complication was, in most patients, no worse than that usually seen with any of the agents administered alone. Only six patients were hospitalized because of persistent nausea and vomiting with or without dehydration. One death was attributed to intractable vomiting and resultant cardiovascular collapse. There was no recorded incidence of drug-induced "flare" in previously radiated areas. The frequency and severity of toxicity among patients who achieved objective response or improvement were similar to those of the group as a whole; 11% had life-threatening toxic effects and 40% sustained maximal toxicity rated as severe. None of the responders had fatal toxic reactions.

DISCUSSION

This study failed to confirm the encouraging response rates reported in the BACON pilot study although nearly half the patients were considered in the most favorable PS category while only one patient of 38 extensive patients in the pilot study was so categorized. What can explain this difference? We believe three factors are likely: (*a*) in the present study, PS was determined prospectively,

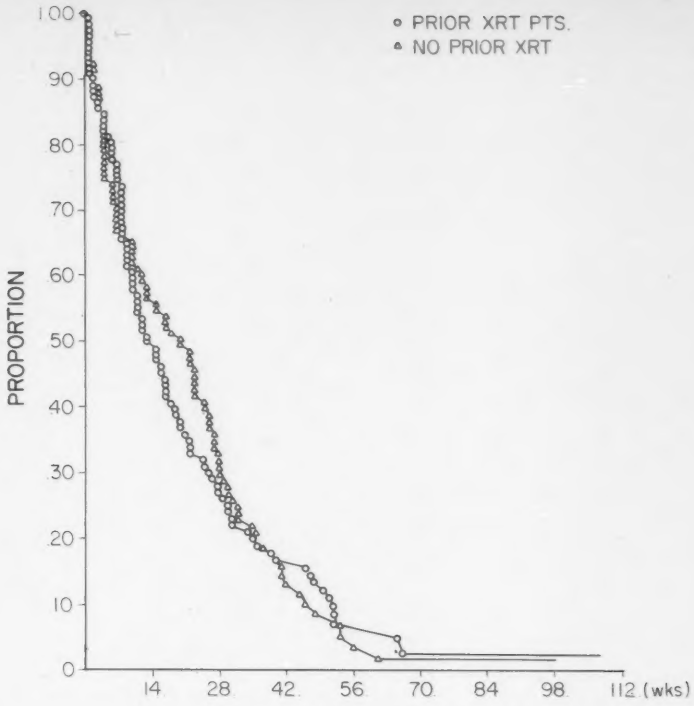

FIGURE 3.—Survival by prior radiotherapy (XRT) status for all patients.

not retrospectively, eliminating the possibility of investigator bias in evaluation "after the fact"; (*b*) twice as many patients in the present study had undergone prior radiation therapy (51% versus 26% in the BACON pilot); (*c*) in spite of our attempts at objectivity, emotional commitment to the pilot therapy may have played a role in the initial evaluations of response. However, the overall response rate among M. D. Anderson Hospital patients was quite similar: 31% in the pilot study and 27% in both the BACON and NAC arms of the present study. Other explanations for the difference in response rate seem less likely; the study chairman was the same, the fractions of patients with life-threatening or fatal (grade 4 or 5) toxic reactions were similar (18% for the pilot and 13% for the group study), granulocyte and platelet count nadirs were similar, and the criteria used to determine response were the same.

The survival results of the present study, within PS categories, are also inferior to the reported results in the BACON pilot: the MST was 9 weeks in PSs 5–7 and 3.5 weeks in PSs 1–4 on the BACON arm of the present study, compared to 20 and 9 weeks, respectively, in the pilot. Again, the most likely explanation appears to lie in the retrospective nature of PS evaluation for the previous study, which led to an underestimation of the "real" pretreatment PS, at least by the standards of other investigators in SWOG. The MST and overall appearance of the survival curves for patients with PSs 5–7 or 1–4 in the present study are indistinguishable from those previously reported by Zelen (5) for patients in the same PS categories who received supportive care alone in the VALCSG study. On the other hand, there may be a modest improvement for fully ambulatory (PSs 8–10) patients, with an MST of 28 weeks with either BACON or NAC and survival to 1 year in approximately 16% (*see* fig 2), compared to an expected MST of 20 weeks and 1-year survival of 10% from the VALCSG supportive care data.

The only subgroup showing evidence of significant benefit from BACON over NAC is the group of fully ambulatory responders, who have a modest but significant prolongation in survival. BACON

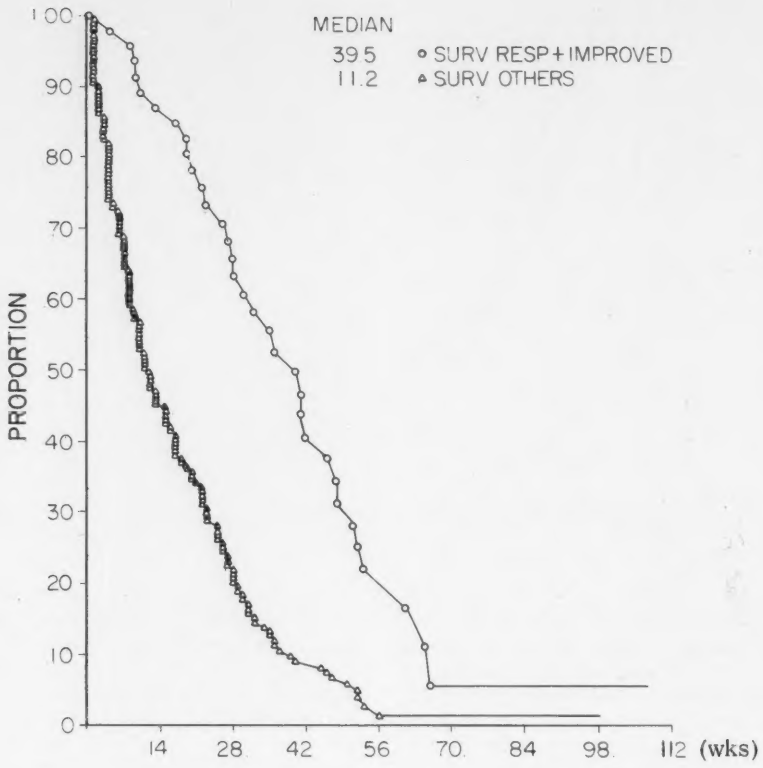

FIGURE 4.—Survival (SURV) by response (RESP) for all patients.

TABLE 3.—Toxicity analysis of 217 patients

	No. of patients (%)	
	BACON	NAC
Evaluable	105	112
Fatal toxicity	4 (4)	4 (4)
Bleomycin, lung	1	0
Sepsis associated with decrease in wbc count	3	3
Vomiting leading to cardiovascular collapse	0	1
Life-threatening toxicity (involved hospitalization)	7 (7)	13 (12)
Granulocytes < 1000/mm³ and fever	4	9
Thrombocytopenia (< 20,000 cells/mm³)	3	4
Severe toxicity	37 (35)	30 (27)
Myelosuppression (< 1000 granulocytes/mm³ and/or < 50,000 platelets/mm³)	26 (25)	21 (19)
Other	11	9
Gastrointestinal	8	7
Bleomycin, skin reaction	1	0
Bleomycin, lung damage (?)	1	0
Weakness	1	0
Adriamycin, paroxysmal atrial tachycardia	0	1
Esophagitis	0	1

actually appears inferior to NAC with respect to patients in the PS 5–7 group (*see* fig 2). Single-drug therapy with a variety of agents has been reported to produce response rates of 20% or more in patients with extensive squamous lung carcinoma (6), but recent experience with alkylating agents (7), nitrosoureas (8), and methotrexate (9), the most commonly used antimetabolite, suggests that the response rate is < 10% for each of them using modern criteria. Adriamycin, however, appears to produce response in approximately 20% of patients, using modern criteria (10,11), and the response duration is comparable to that which we observed in the present study.

The data are at present inadequate to make a statement about the effect of adriamycin alone in a similar group.

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Effects of Variations in Renal Function on the Clinical Pharmacology of Bleomycin Administered as an Iv Bolus [1,2]

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SUMMARY

The clinical pharmacology of bleomycin administered as an iv bolus has been studied in a homogeneous group of patients receiving a single regimen containing bleomycin, vinblastine, and *cis*-dichlorodiammineplatinum(II). These studies demonstrated that in patients with creatinine clearances ≥ 35 ml/minute, the serum (or plasma) terminal elimination half-life of bleomycin was approximately 115 minutes. In patients with creatinine clearances < 25–35 ml/minute, the terminal elimination half-life increased exponentially as the creatinine clearance decreased. The volume of distribution was approximately 20 liters, and was unaffected by changes in the creatinine clearance. The microbiologic assay and radioimmunoassay employed gave equivalent results.

[Cancer Treat Rep 61:1631–1636, 1977]

Bleomycin is an antitumor antibiotic isolated from *Streptomyces verticillus* (1). It has been shown to be active in squamous cell carcinomas, lymphomas, and testicular carcinomas, and its most significant toxicity has been shown to be pulmonary fibrosis (2,3). It has been suggested that bleomycin induces its antitumor effects by producing single and double stranded breaks in preformed DNA (4).

Until recently, little information concerning the clinical pharmacology of bleomycin has been available. Initial clinical pharmacokinetic studies employed a microbiologic assay using a *Bacillus subtilis* (*B. subtilis*) ATCC-6633 spore solution which was sensitive to approximately 0.2 milliunit/ml (5). The elimination of bleomycin from the blood was reported to vary, with some patients demonstrating first order kinetics and others not. The plasma half-life was reported to vary from 16 to 45 minutes at doses > 26 units/m^2 in one study (6). In another study, a dose of 15 units was reported to result in a maximum blood level of 3.3 μg/ml and a serum half-life of 14 minutes (7).

Previous reports concerning the clinical pharmacology of bleomycin included data from a very small number of patients for whom no data were presented relative to creatinine clearance (5,6). Furthermore, it was not totally clear in either publication whether $t_{\frac{1}{2}}\alpha$ or $t_{\frac{1}{2}}\beta$ was determined.

Recently, a radioimmunoassay for bleomycin was developed which proved to be more sensitive than previously reported microbiologic assays (8). Previous studies have demonstrated that the antiserum used in the radioimmunoassay is quite specific since even minor modifications of the bleomycin molecule resulted in marked decreases in cross reactivity to antiserum (9). Previous studies also demonstrated that within the range of sensitivity of the microbiologic assay, the radioimmunoassay and microbiologic assay give equivalent results in both serum and plasma (10).

The studies described in this paper were designed (*a*) to determine the pharmacokinetics of bleomycin

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administered to patients as an iv bolus, (*b*) to determine the effects of varying renal function on the clinical pharmacokinetics of bleomycin, and (*c*) to compare the data obtained with the radioimmunoassay to those obtained using the microbiologic assay.

MATERIALS AND METHODS

Assay Procedures

The microbiologic assay was performed using a *B. subtilis* ATCC-6633 spore solution as previously described (5). The radioimmunoassay was performed as previously described (8). Replicates of each concentration were performed, and the results were found to be readily reproducible. Each sample was analyzed by both methods in a blinded fashion. After completion of the determinations, the results of each assay were compared.

Sample Collection

Venous and arterial blood specimens were obtained in heparinized tubes at specified intervals up to 72 hours after a dose of bleomycin. When serum was to be assayed, blood specimens were collected in nonheparinized tubes. Specimens were refrigerated and centrifuged, and either plasma or serum was obtained and stored frozen (–20°C) until analyzed. Urine specimens were collected for varying periods of time, stored on ice, and frozen immediately at the termination of the collection period.

Blood and urine for pharmacologic studies were obtained from patients being treated with bleomycin, vinblastine, and *cis*-dichlorodiammineplatinum (II) after the dose of bleomycin and approximately 72 hours after completion of the *cis*-dichlorodiammineplatinum(II) infusion. Creatinine clearance was determined on the day prior to or the same day as bleomycin administration.

Patient Selection

To minimize variables, all patients enrolled in the pharmacokinetics study had disseminated testicular carcinomas and were treated with bleomycin (30 units/week × 12 weeks), vinblastine (0.2 mg/kg/day × 2 every 3 weeks), and *cis*-dichlorodiammineplatinum(II) (20 mg/m², Days 1–5 every 3 weeks for 9 weeks) according to the regimen developed by Einhorn et al (11). One patient (No. 1) received 15 units of bleomycin instead of 30 units. These patients were selected because of the relative homogeneity of this group of patients and to obviate potential pharmacokinetic differences due to drug interactions occurring in patients treated with varying regimens.

Additionally, since a major goal of the study was to determine the effect of varying renal function on bleomycin pharmacokinetics, the nephrotoxicity of this regimen allowed evaluation of patients in whom creatinine clearance varied during the study, and resulted in a more rapid accrual of patients with varying creatinine clearances than was possible with other regimens.

Pharmacologic Calculations

NONLIN computer analysis was used for the treatment of pharmacokinetic data. The method used is an adaptation of Hartley's modification of the Gauss-Newton method for linearizing equations and applying linear least squares (12). Each set of data for each patient was computer fitted, and the best fit curve, ie, one, two, or three exponential curves, was determined. In all cases, the curve of best fit was the biexponential function.

RESULTS

Table 1 shows the characteristics of patients enrolled in this study. The median age was 24 years with ages varying from 18 to 47 years. With two exceptions, all initial performance statuses (Karnofsky scale) were ≥ 60%. Initial creatinine clearances varied from 10.7 to 180 ml/minute corrected to 1.73 m² of body surface area. All evaluable patients achieved either a partial (> 50% decrease in the cross-sectional area of measurable lesions) or complete response. Two patients were not evaluable for response because of lack of measurable disease.

In a previous study (10), plasma and serum bleomycin concentrations were shown to be equivalent when determined by either the radioimmunoassay or the microbiologic assay. In this study, the coefficient or correlation between the microbiologic assay and radioimmunoassay was 0.99, as previously reported, and plasma and serum concentrations were found to be equivalent. Thus, serum and plasma values are used interchangeably.

Table 2 presents the plasma (serum) $t_{\frac{1}{2}}\beta$ and the area under the curve (AUC) of bleomycin determined by the microbiologic assay and radioimmunoassay relative to creatinine clearance. In general, the data shown in table 2 demonstrate that the plasma $t_{\frac{1}{2}}\beta$ of bleomycin determined by both methods was similar, and that the plasma clearance of bleomycin varied with creatinine clearance. The values for AUC were variable and did not correlate well with the creatinine clearance or $t_{\frac{1}{2}}\beta$. This may be explained by slight variations in the timing of

TABLE 1.—Patient characteristics

Patient No./age	m^2	Performance status (%)	Initial creatinine clearance (ml/min)	Site of metastases	Tumor response*
1/24	2.0	90	10.7	Pulmonary	CR
2/33	1.95	30	21.3	Pulmonary, central nervous system	PR
3/29	2.3	80	62	Left inguinal node	CR
4/25	1.8	80	64.6	Left cervical node, para-aortic	PR
5/27	1.65	90	78	Liver	PR
6/46	1.6	80	88	Lung	PR
7/21	2.3	80	89	Bone, lung, retroperitoneal nodes	PR
8/24	1.9	60	98.6	Retroperitoneal mass	PR
9/20	1.9	90	98.7	Pelvis, mediastinum, para-aortic	PR
10/47	2.1	90	107	Pulmonary, mediastinum	PR
11/22	1.5	90	108.5	Skull, shoulder, elbow	NE
12/22	2.1	90	109	Pulmonary, lymph node	CR
13/25	1.76	90	110	Adjuvant therapy	NE
14/26	2.08	90	115	Mediastinal mass	PR
15/26	1.68	90	118	Pulmonary	CR
16/26	1.88	90	123	Lung, retroperitoneal nodes	CR
17/22	2.1	90	128	Large retroperitoneal mass, lung	PR
18/18	1.84	90	140	Mediastinal mass, pulmonary	CR
19/27	1.7	100	157	Lung	PR
20/22	1.98	90	180	Pulmonary, liver, para-aortic	PR
Median 24	1.9				

*CR = complete response; PR = partial response; NE = not evaluable.

TABLE 2.—Plasma $t_i\beta$ of bleomycin vs creatinine clearance

Patient No.	Creatinine clearance (ml/min)	Microbiologic assay		Radioimmunoassay	
		t_i (mins)	AUC	t_i (mins)	AUC
1	10.7	1260	—	1260	—
1	15.2	660	—	660	—
2	21	375	255	341	452
11	35	276	670	178	480
7	48	119	172	—	—
3	62	157	378	114	309
4	65	171	532	177	256
7	89	158	210	117	142
15	98	129	160	150	318
8	99	92	159	68	141
9	99	114	168	133	350
12	105	123	173	103	249
10	107	100	71	106	146
11	108	41	74	25	145
12	109	121	72	86	146
13	110	85	217	74	292
14	115	94	73	117	155
15	118	173	149	129	257
7	120	63	137	79	188
20	122	134	209	209	270
16	123	114	155	103	184
14	127	115	139	98	191
18	140	112	183	98	189
18	146	121	218	98	270
20	149	114	137	124	218
20	180	95	89	140	185

the first blood sample relative to the time of injection of bleomycin. Since patient 1 received only 15 units of bleomycin, AUC data are not presented.

Figure 1 shows a semi-logarithmic plot of the plasma $t_{\frac{1}{2}}\beta$ versus the creatinine clearance. This figure demonstrates that the inflection point, ie, the point at which the plasma $t_{\frac{1}{2}}\beta$ of bleomycin becomes prolonged, is at a creatinine clearance of approximately 25–35 ml/minute. At creatinine clearances > 25–35 ml/minute, the mean $t_{\frac{1}{2}}\beta$ was 122.7 ± 9.6 minutes when determined with the microbiologic assay and 115.4 ± 8.6 minutes when determined with the radioimmunoassay. Below a creatinine clearance of approximately 25–35 ml/minute, the plasma $t_{\frac{1}{2}}\beta$ of bleomycin increased exponentially as the creatinine clearance decreased. The mean $t_{\frac{1}{2}}\beta$ in patients with creatinine clearances > 70 ml/minute did not differ from the mean of patients with creatinine clearances > 25–35 ml/minute (111 or 109 minutes as determined by the microbiologic assay or radioimmunoassay respectively). Equivalent data were obtained when the radioimmunoassay was employed.

Bleomycin pharmacokinetics were evaluated sequentially in eight patients (table 3). These studies demonstrate that, within a given patient, the plasma half-life of bleomycin varied inversely with the creatinine clearance.

Table 4 shows that the total apparent volume of distribution varied from 14 to 35 liters except for two patients who had volumes of distribution of 6.5 and 8.3 liters. The mean volume of distribution of bleomycin was 22.7 ± 6.02 and 19.8 ± 1.46 liters in patients with creatinine clearances greater or less than 35 ml/minute respectively.

Table 5 shows the urinary excretion of bleomycin relative to creatinine clearance. Approximately 50%

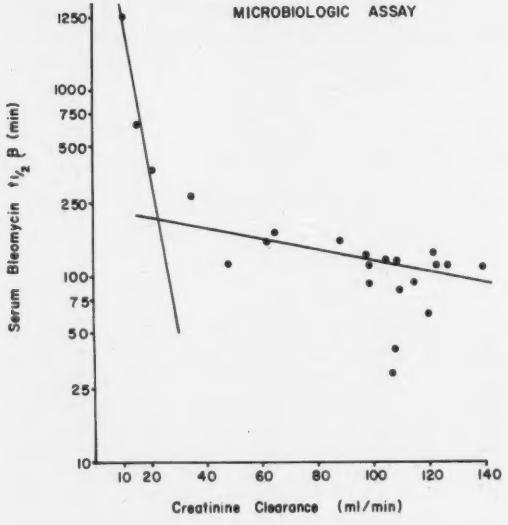

FIGURE 1.—Semi-logarithmic plot of serum $t_{\frac{1}{2}}\beta$ of bleomycin vs creatinine clearance from values determined using the microbiologic assay.

TABLE 3.—Changes in renal status and plasma $t_{\frac{1}{2}}\beta$ of bleomycin

Patient No.	Wk of therapy	Creatinine clearance (ml/min)	$t_{\frac{1}{2}}$ (mins)
15	0	118	129
15	5	98	150
14	0	115	117
14	6	127	98
18	0	140	98
18	6	146	98
12	0	109	86
12	5	105	103
20	0	180	95
20	6	149	114
20	9	122	134
11	0	108	41
11	9	35	276
7	0	89	158
7	3	120	63
7	6	48	119
1	0	10.7	1260
1	1	15.2	660

TABLE 4.—Creatinine clearance vs volume of distribution

Patient No.	Creatinine clearance (ml/min)	Volume of distribution (liters)
1	15	34.8
2	21	16.3
11	35	17.2
7	48	33.1
3	62	13.9
4	65	6.5
7	89	29.4
15	98	18.8
9	99	14.5
8	99	19.1
12	105	15.8
10	107	23.6
11	108	16.3
12	109	22.9
13	110	8.3
14	115	30.1
15	118	19.8
7	120	18.1
20	122	26.7
16	123	29.4
14	127	19.7
18	140	18.8
18	146	13.6
20	149	23.4
20	180	28.6

TABLE 5.—Bleomycin excretion vs creatinine clearance

Patient No.	Creatinine clearance (ml/min)	$t_{\frac{1}{2}}\beta$ (mins)	Cumulative % of bleomycin excreted in urine			
			0–4 hrs	0–8 hrs	0–12 hrs	0–24 hrs
7	48	119	50	58.3	59.9	60.6
3	62	157	29	40.6	46.2	53.8
4	65	171	35.3	46.9	52.9	58.2
7	89	158	53	69.0	71.3	75.6
15	98	150	54	70.0	75.0	77.6
12	105	103	57.3	63.3	67.9	69.5
14	115	117	62.6	76.6	80.6	82.1
20	122	134	50	62.0	70.3	70.3
18	140	98	47.6	63.6	66.4	67.9
Mean	93.7±10.1	134±8.7	48.7±3.5	61.1±3.7	65.6±3.6	68.4±3.1

(mean) of the total dose was excreted during the first 4 hours after the dose was given. Within 24 hours after the dose, approximately 70% of the total dose was excreted in the urine.

DISCUSSION

The conclusions derived from these and the previously reported studies (10) are: (*a*) bleomycin is cleared principally by renal mechanisms; (*b*) at creatinine clearances ≥ 25–35 ml/minute, the $t_{\frac{1}{2}}\beta$ of bleomycin is approximately 115 minutes; (*c*) at creatinine clearances < 25–35 ml/minute the $t_{\frac{1}{2}}\beta$ increases exponentially as the creatinine clearance decreases; (*d*) even in frank renal failure, no bleomycin was detectable in the blood 48–72 hours after a bolus injection; (*e*) in patients with creatinine clearances ≥ 50 ml/minute, approximately 50% of a total dose of bleomycin is excreted in the urine in 4 hours and > 70% in 24 hours; (*f*) the mean total apparent volume of distribution is approximately 20 liters and does not vary with the creatinine clearance; (*g*) bleomycin is probably not dialyzable; (*h*) the pharmacokinetic model describing bleomycin pharmacokinetics does not vary with varying renal function, ie, a two-compartment model best explains the data in all patients; and (*i*) both the microbiologic assay and radioimmunoassay give comparable results in plasma or serum.

It is unlikely that the $t_{\frac{1}{2}}\beta$ of bleomycin determined in this study is artificially prolonged due to transient decreases in renal function associated with administration of *cis*-dichlorodiammineplatinum(II) since the creatinine clearances were determined during or on the day immediately prior to the day samples for bleomycin pharmacokinetics were obtained, and approximately 72 hours after completion of the *cis*-dichlorodiammineplatinum(II) dosing.

These studies demonstrate that the likelihood of plasma accumulation of bleomycin is minimal in patients with creatinine clearances > 25–35 ml/minute when bleomycin is administered on a twice weekly schedule. Moreover, in a patient in frank renal failure no bleomycin was detectable in the blood 48–72 hours after administration, suggesting that plasma accumulation may also not occur on a twice weekly schedule even in patients with severe renal dysfunction. However, additional data are necessary prior to reaching a firm conclusion regarding patients with a creatinine clearance < 35 ml/minute, and definite recommendations concerning dosage adjustments await the completion of a study in which variations in the incidence and severity of toxicities are determined relative to variations in renal function.

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Pharmacokinetics of the Absorption, Distribution, and Elimination of Melphalan in the Dog [1,2,3]

R. L. Furner,* R. K. Brown, and G. Duncan [4,5]

SUMMARY

[^{14}C]melphalan ([^{14}C]L-PAM) was rapidly absorbed from the gut of dogs after oral dosing and reached a maximum concentration in the serum by 30 minutes. The disappearance of L-PAM (intact drug) from the serum was biphasic after iv administration, with half-lives of 14 and 66 minutes for the α and β phases, respectively. The urinary excretion accounted for 44% of the total radioactivity and 25% appeared in the feces. Approximately 8% of the dose was excreted unchanged in the urine. Biliary excretion was rapid, with 11% of the dose being accounted for in the bile after 30 minutes; approximately 80% of these drug equivalents (materials containing radioactivity) was parent compound. Since large amounts of L-PAM appeared in the bile, the agent may prove to be active against cancers of the gall bladder, bile ducts, and duodenum.

[Cancer Treat Rep 61:1637–1646, 1977]

Although melphalan (L-PAM) has been available for 25 years, little is known of its biologic distribution, metabolism, and excretion. We recently reported the development of a method (1) by which unlabeled L-PAM could be separated from its metabolites and products of hydrolysis for quantitative analysis. We report here studies in dogs on the absorption, distribution, excretion, and degradation of [^{14}C]L-PAM. A preliminary report of this work has appeared (2).

MATERIALS AND METHODS

Melphalan.—[^{14}C]L-PAM was labeled in the mustard sidechain. Purification was accomplished by dissolving the drug in 11 mM HCl (1 mg/ml) and injecting 1 ml into the high-pressure liquid chromatograph (described below). Fractions containing L-PAM were collected and made acid with 3 N HCl. The fractions were evaporated to dryness under vacuum, and the purified L-PAM was taken up in 11 mM HCl in ethanol. Purity averaged 95% for L-PAM. The monohydroxy derivative accounted for 5% of the total. Specific activity was 19.8 microcuries/mg (6.04 microcuries/mM). Dosing solutions were prepared by evaporating portions of the ethanolic mixture to dryness and adding an appropriate amount of unlabeled L-PAM. The preparation was dissolved in 11 mM HCl immediately prior to injection. Specific activity and purity of each dosing solution were assessed (1). Each dog received approximately 25 microcuries of radioactivity (0.45 mg/kg or 9.0 mg/m^2) orally or iv.

Animals.—Female beagle dogs were obtained from the colony at Southern Research Institute. A preliminary examination of their blood chemistries and hematologies indicated that the animals were in good health. Food was withheld for 18 hours prior to dosing. Indwelling, Foley catheters were used to facilitate the collection of urine. A control sample of blood was obtained by venipuncture immediately prior to dosing. In the recovery study, the urinary catheters were removed after 12 hours; thereafter, the urine was collected from "catch-

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[5]We appreciate the assistance of Dr. Harris Lloyd and Mr. Henry Finch in setting up and running the necessary computer programs used in the calculation of the pharmacokinetic parameters described herein.

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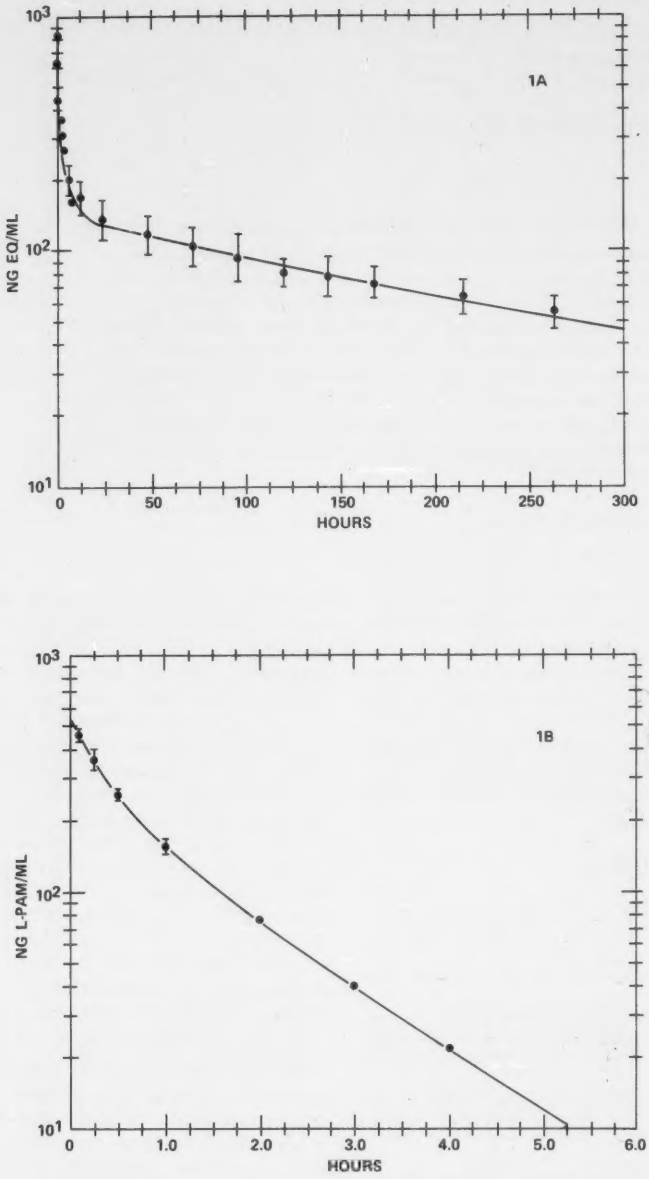

FIGURE 1.—A. Serum levels of drug equivalents of [^{14}C]L-PAM after iv dosing. Female beagle dogs weighing 8–10 kg were dosed iv with [^{14}C]L-PAM (0.45 mg/kg in 11 mM HCl). Data are means ± SE from 2–6 animals for each time point. B. Serum levels of [^{14}C]L-PAM after iv dosing (0.45 mg/kg). Data represent 1–4 animals for each time point.

pans" of individual metabolism cages. In the distribution study, final blood and urine samples were collected, and then the animals were anesthetized with ether and killed by exsanguination. All tissue, urine, and fecal samples were placed on ice immediately after collection to minimize the degradation of L-PAM and its products. Samples were assayed for total radioactivity via sample oxidation and/or high-pressure liquid chromatography. Remaining portions of samples and the carcass were frozen at -20°C pending further use.

Sample oxidation.—Duplicate samples were weighed to the nearest milligram and combusted in a Packard 306 sample oxidizer. Carbon dioxide was trapped using Carbosorb which was mixed with Permafluor V (Packard Instruments Co, Downers Grove, Ill) to form the scintillation cocktail. Samples were counted in a Packard scintillation counter equipped with an external standard. Variation between duplicate samples was generally < 7%. Samples that were not obvious duplicates were re-analyzed using portions of the tissue or fluid that had been frozen and retained for that purpose. The data are averages of those duplicates.

High-pressure liquid chromatography.—To assess the amount of parent compound present, we extracted portions of selected tissues or fluids with a mixture of 10% perchloric acid in 2-methoxyethanol:0.1% acetic acid (55:45, vol/vol). After neutralization of the acid and filtration through a millipore filter as described previously (1), the extract was injected into a liquid chromatograph equipped with two pumps, a solvent flow programmer, a μBondapak C_{18} column, and an ultraviolet detector (all from Waters Associates, Bedford, Mass).

Solvent programming was initiated immediately after injection of the sample. The initial solvent composition was 2-methoxyethanol:0.1% acetic acid (16.8:83.2, vol/vol) which was formed by mixing 85% solvent A (2-methoxyethanol:0.1% acetic acid [10:90, vol/vol]) with 15% solvent B (2-methoxyethanol:0.1% acetic acid [55:45, vol/vol]). After 3 minutes, the step gradient changed the solvent composition to 49.3:51.7, vol/vol, 2-methoxyethanol:0.1% acetic acid, which was formed from 15% solvent A and 85% solvent B. The flow rate across the column was 1.5 ml/minute.

Fractions of 0.25 or 0.5 ml were collected in scintillation vials and mixed with Aquasol (New England Nuclear Corp, Boston, Mass). Efficiency of scintillation counting was approximately 80%.

Pharmacokinetics.—Rate constants and half-lives for total drug equivalents were estimated by fitting a poly-exponential equation to data through the use of a nonlinear least squares program, NONLIN, developed by the Biomathematics Unit of the Upjohn Company (Kalamazoo, Mich) (3). Initial parameter estimates were obtained by exponential stripping (4). The data were automatically plotted in a format which displayed the average ± SE of the data points and the curve was generated by the computer to fit the poly-exponential equation. The area under the curve (C × t) was also calculated.

RESULTS

Elimination of L-PAM and L-PAM Equivalents From Serum and Whole Blood

Drug equivalents (materials containing radioactivity) derived from [^{14}C]L-PAM disappeared from both serum and whole blood in three exponential phases after iv dosing (fig 1A). The concentration of drug equivalents was 20% higher in serum than in whole blood at early time periods after dosing. The disappearance of L-PAM from serum was biphasic through 4 hours after iv dosing (fig 1B). Further observations were beyond the limits of sensitivity of the assay.

After oral dosing of dogs, L-PAM was rapidly absorbed from the gut, with the maximum serum concentration of drug equivalents occurring at approximately 30 minutes (fig 2A). Elimination of drug equivalents from the serum followed a pattern similar to the pattern observed after iv dosing. L-PAM was analyzed in the serum of a single dog after oral dosing (fig 2B). Peak serum levels were attained at 30 minutes; only a single phase of elimination was observed.

The pertinent pharmacokinetic parameters derived from these experiments are shown in table 1.

Elimination of L-PAM and L-PAM Equivalents in Urine and Feces

The rate of excretion of drug equivalents derived from [^{14}C]L-PAM declined in a triphasic manner after iv or oral dosing (fig 3A and B). About 44% of the dose administered was excreted in the urine in 11 days; 22% of the dose was excreted within 4 hours. Total urinary excretion appeared to be independent of the route of drug administration, but the rates of urinary excretion differed slightly, as indicated in table 2. During the first hour after dosing, 15%–25% of the drug equivalents excreted was L-PAM. Thereafter, L-PAM comprised no more than 9% of the drug equivalents excreted per hour. Since the data available were not extensive enough

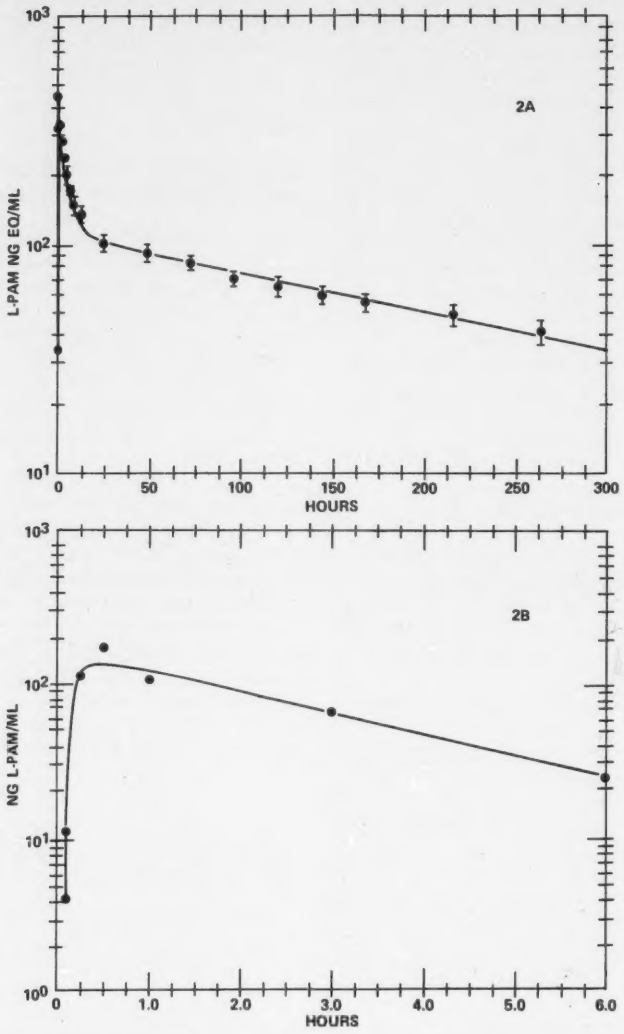

FIGURE 2.—A. Serum levels of drug equivalents of [^{14}C]L-PAM after oral dosing. Female beagle dogs weighing 8–10 kg were dosed orally with [^{14}C]L-PAM (0.45 mg/kg in 11 mM HCl). Data points are the mean ± range for 2 animals at each time period. B. Serum levels of [^{14}C]L-PAM after oral dosing (0.45 mg/kg). Data are from a single animal.

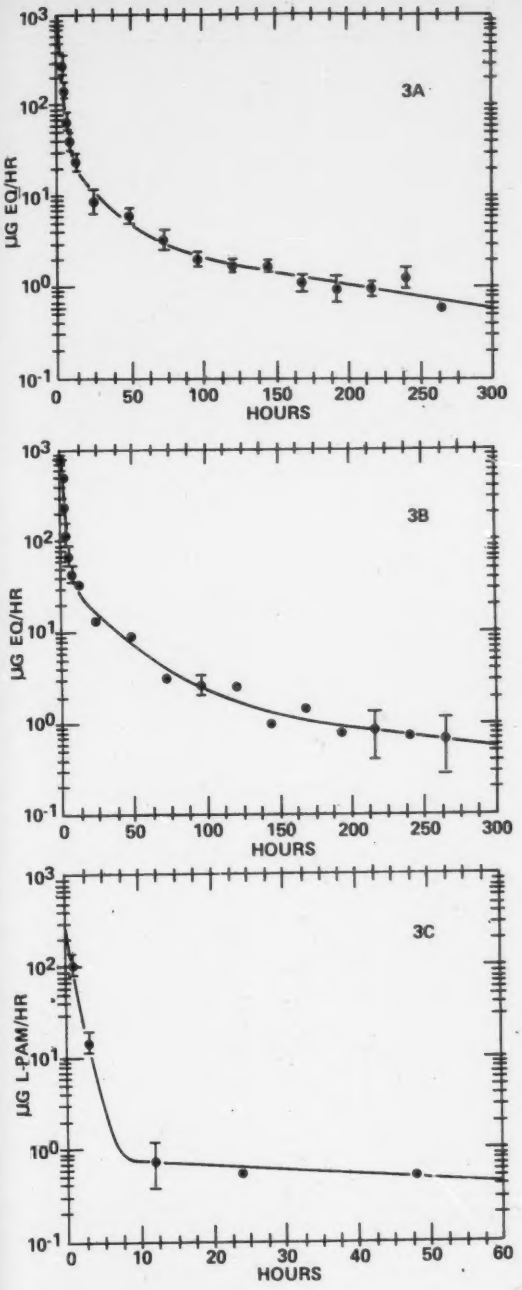

FIGURE 3.—A. Urinary excretion of drug equivalents of [^{14}C]L-PAM after iv dosing. Female beagle dogs weighing 8–10 kg were dosed iv with [^{14}C]L-PAM (0.45 mg/kg in 11 mM HCl). Data are means ± SE from 1–6 animals for each time point. B. Urinary excretion of drug equivalents of [^{14}C]L-PAM after oral dosing (0.45 mg/kg). Data are means ± range from 2 dogs. C. Urinary excretion of [^{14}C]L-PAM after oral or iv dosing (0.45 mg/kg). Data are means ± SE from 1–3 dogs at each time period. A total of 7 dogs were used.

TABLE 1.—Pharmacokinetic parameters derived from measurement of [^{14}C]L-PAM or drug equivalents in serum and whole blood of dogs given 0.45 mg/kg of [^{14}C]L-PAM*

Parameter	Serum		Whole blood
	[^{14}C]L-PAM	Drug equivalent	Drug equivalent
	iv (5)†	*iv (6)*†	*iv (2)*†
α (hr^{-1})	2.9 ± 0.8‡	5.6 ± 1.9§	2.6 ± 0.8§
β (hr^{-1})	0.62 ± .049	0.25 ± 0.05	0.16 ± 0.07
γ (hr^{-1})	—	0.0038 ± 0.0005	0.0023 ± 0.0005
$t_{\frac{1}{2}\alpha}$ (hr)	0.24	0.12	0.27
$t_{\frac{1}{2}\beta}$ (hr)	1.10	2.80	4.30
$t_{\frac{1}{2}\gamma}$ (hr)	—	180	300
A_0 (ng/ml)	270 ± 37	590 ± 130	520 ± 110
B_0 (ng/ml)	260 ± 38	320 ± 40	140 ± 40
C_0 (ng/ml)	—	140 ± 11	120 ± 9
C × t (ng-hr/ml)	520¶	37,000	46,900
R^2	0.999**	0.993	0.991
	Oral (1)†	*Oral (2)*†	*Oral (2)*†
k_a (hr^{-1})	8.4 ± 6.8††	9.8 ± 4.5‡‡	7.2 ± 3.4‡‡
α (hr^{-1})	0.32 ± 0.15	0.27 ± 0.06	0.49 ± 0.13
β (hr^{-1})	—	0.0040 ± 0.0006	0.0030 ± 0.0006
$t_{\frac{1}{2}k_a}$ (hr)	0.083	0.07	0.09
$t_{\frac{1}{2}\beta}$ (hr)	2.2	2.60	1.50
$t_{\frac{1}{2}\gamma}$ (hr)	—	180	230
A_0 (ng/ml)	−310 ± 120	−880 ± 270	−590 ± 110
B_0 (ng/ml)	170 ± 50	320 ± 50	240 ± 60
C_0 (ng/ml)	—	110 ± 10	96 ± 7
C × t (ng-hr/ml)	500	28,400	30,700
R^2	0.998	0.989	0.980

*Values are $\bar{X}$ ± SD.

†Nos. in parentheses = total No. of animals in each series.

‡Data fitted to a biexponential equation of the form $C = A_0e^{-\alpha t} + B_0e^{-\beta t}$.

§$C = A_0e^{-\alpha t} + B_0e^{-\beta t} + C_0e^{-\gamma t}$ where C is the concentration at any time, t. A_0, B_0, and C_0 are the zero time intercepts for the phases of elimination and α, β, and γ are rate constants describing the decay of those phases of elimination.

$$C \times t = \int_0^\infty Cdt.$$

$$**R^2 = \frac{\text{weighted corrected sum of squared observations} - \text{sum of weighted squared deviations}}{\text{weighted corrected sum of squared observations}}.$$

††Data fitted to a biexponential equation of the form $C = A_0e^{-k_a t} + B_0e^{-\alpha t}$ where k_a is the absorption constant.

‡‡Data fitted to a triexponential equation of the form $C = A_0e^{-k_a t} + B_0e^{-\alpha t} + C_0e^{-\beta t}$ where k_a is the absorption constant.

TABLE 2.—Pharmacokinetic parameters derived from measurement of [^{14}C]L-PAM or drug equivalents in urine and feces of dogs given 0.45 mg/kg of [^{14}C]L-PAM*

Parameter	Urine			Feces	
	Drug equivalents		[^{14}C]L-PAM	Drug equivalents	
	iv (6)†	Oral (2)†	iv + oral (7)†	iv (2)†	Oral (2)†
α (hr^{-1})	0.53 ± 0.06‡	0.42 ± 0.08‡	1.00 ± 0.17§	0.038 ± 0.004¶	0.022 ± 0.005¶
β (hr^{-1})	0.048 ± 0.016	0.038 ± 0.011	0.011 ± 0.012	—	—
γ (hr^{-1})	0.0059 ± 0.0020	0.0048 ± 0.0033	—	—	—
A_0 (μg/day)	1000 ± 150	510 ± 120	310 ± 120	92 ± 46**	23 ± 19**
B_0 (μg day)	27 ± 9	35 ± 13	0.85 ± 0.33**	—	—
C_0 (μg/day)	3.4 ± 1.3**	2.4 ± 1.7**	—	—	—
Average dose (μg/dog)	4800	4400	4600	4800	4400
Average μg equivalent excreted	2200	1900	—	1200	1120
% of dose excreted	45	43	8	24	26

*Values are $\bar{\chi}$ ± SD.

†No. of animals used.

‡Data fitted to a triexponential equation of the form $V = A'_0e^{-\alpha t} + B'_0e^{-\beta t} + C'_0e^{-\gamma t}$ where V is the rate of excretion at any time, t. A'_0, B'_0, and C'_0 are zero time intercepts describing apparent initial rates of elimination and α, β, and γ are constants describing the rate of decline in the rate of elimination.

§Data fitted to a biexponential equation of the form $V = A'_0e^{-\alpha t} + B'_0e^{-\beta t}$.

¶Data fitted to a monoexponential equation of the form $V = A'_0e^{-\alpha t}$.

**The use of additional exponential terms did not result in a reduction in the sum of weighted squared deviations; $R^2 = 1.00$ in all of the above calculations (*see* table 1).

to permit formal statistical analysis of both groups for comparison, the data were pooled in an attempt to demonstrate the general characteristics of elimination of parent compound. The rate of excretion of parent compound declined in a biphasic fashion (fig 3C). The overall elimination rate constant for parent compound was 0.071 hour^{-1}. Expression of the data for urine and fecal elimination in the form of micrograms/hour versus time permitted the derivation of rate constants without the requirement that samples at every time period be analyzed.

The rate of fecal excretion of drug equivalents after iv dosing declined as a first-order process during the first 5 days. Although radioactivity was detected in the feces through 11 days, the amount eliminated in the feces each day remained nearly the same after 5 days. The model was not improved through the use of two exponential terms.

After oral doses to dogs, rate constants for fecal elimination could not be determined. However, 22% of the dose administered was excreted in the feces in 5 days; 25% was excreted within 11 days.

Distribution

Tissues, tissue contents, and fluids were assayed for their content of radioactivity derived from [^{14}C]L-PAM (table 3).

Thirty minutes after iv dosing with [^{14}C]L-PAM, the concentrations of [^{14}C]L-PAM equivalents were higher in liver, gall bladder, bile, kidney, urinary bladder, spleen, stomach contents, and duodenum contents than in serum. After 4 hours the concentration of drug equivalents had declined in the serum by 34%, in the liver by 73%, in the kidney cortex by 31%, and in the kidney medulla by 77%. In the same time interval, the concentration of drug equivalents increased in gall bladder, bile, and contents of small intestine.

Analysis of selected samples revealed that the parent compound, L-PAM, represented 63% of the total drug equivalents present in serum at 30 minutes after dosing; after 4 hours, L-PAM comprised only 8% of the drug equivalents in serum. L-PAM comprised 86% of the drug equivalents found in bile after 30 minutes and 72% after 4 hours. In the liver, only 2% of the total drug equivalents was in the form of L-PAM after 30 minutes, and after 2 hours, no parent compound was detected in the liver.

DISCUSSION

Although L-PAM has been used clinically for a number of years in the treatment of various neoplasms (5), little was known of the uptake, distribution, metabolism, and pharmacokinetics of the drug until the appropriate techniques were developed for identifying and quantifying the parent compound in extracts of biologic materials (1).

The work by Milner et al (6) was accomplished using [^{3}H]L-PAM in rats and indicated a biphasic

TABLE 3.—Distribution of drug equivalents of L-PAM in dogs at various times after dosing with [^{14}C]L-PAM, 0.45 mg/kg, iv

Tissue, tissue contents, fluids, or solids	Drug equivalent (ng/g) at—*			
	30 mins†	1 hr†	2 hrs†	4 hrs†
Serum	422 (63%)	374 (44%)	301 (26%)	280 (8%)
Liver	2190 (2%)	2020 (1%)	1090 (0)	591 (0)
Gall bladder	3180	5460	15,100	11,900
Bile	52,400 (86%)	93,100 (83%)	65,000 (83%)	123,000 (72%)
Kidney (cortex)	1330 (29%)	1820 (13%)	1030 (13%)	925 (2%)
Kidney (medulla)	2130	5270	2210	488
Urinary bladder	3640	3780	2310	1520
Skeletal muscle	250	338	219	210
Heart	438	442	314	275
Lung	465	437	333	286
Spleen	537 (40%)	288 (40%)	403 (11%)	287 (5%)
Bone	22	37	11	24
Bone marrow	73	85	23	96
Brachial lymph node	—	240	334	162
Mesenteric lymph node	288	—	—	—
Eye (solids)	164	186	123	134
Eye (liquids)	17	27	21	29
Cerebrum	70	74	34	65
Cerebellum	74	94	36	81
Medulla	46	44	30	72
Brachial nerve	100	94	44	126
Skin	151	187	163	196
Mammary fat	180	129	90	225
Retroperitoneal fat	154	59	18	62
Diaphragm	317	349	303	266
Pancreas	360	292	219	224
Adrenal	294	260	181	192
Thyroid	236	241	220	158
Ovary	310	310	272	254
Pituitary	328	301	255	233
Submaxillary	457	434	321	542
Parotid	280	260	176	194
Stomach	337	313	267	219
Stomach contents	1210	53	4200	39
Duodenum	458	1360	378	519
Duodenum contents	1180	30,200	2180	4230
Jejunum	351	1000	1100	457
Jejunum contents	454	8200	4730	7270
Ileum	321	306	1390	266
Ileum contents	406	408	50,700	25,510
Cecum	298	328	324	241
Cecum contents	131	116	143	97
Colon	314	294	265	248
Colon contents	26	16	24	6
Thymus	238	101	144	—

*Total drug equivalents were determined via sample oxidation followed by liquid scintillation counting.

†Time of sacrifice postinjection; 1 dog at each time period.

‡Nos. in parentheses = % of the total represented by L-PAM; analysis was by high-pressure liquid chromatography.

disappearance of drug equivalents from plasma with half-lives of about 24 minutes and 6 hours for the distribution and elimination phases. The data from Cohn (7) suggested apparent half-lives for total radioactivity of approximately 3.3 and 66 hours for the distribution and elimination phases, respectively. Studies in our laboratory using unlabeled drug revealed a single phase of elimination for parent compound with a half-life of 41 minutes in mice (1).

In this paper we have described triphasic elimination of drug equivalents from the serum of dogs with half-lives of 7.8 minutes, 2.8 hours, and 180 hours. The data of Milner et al (6) and of Cohn (7) are not strictly comparable since those investigators did not collect samples at the early time points

described here. However, these investigators did foresee considerable differences in the rate of drug elimination at early versus later time periods after dosing. Our data indicate that the parent compound is eliminated in two phases with half-lives of 14 and 66 minutes, respectively. These data are in reasonable agreement with our previous report of apparent half-lives of 29 and 51 minutes, respectively. It is worth noting that the doses administered in the two studies differed by a factor of 10.

The dose administered in the current study was chosen to approximate clinically used doses, but using this low dose, the limits of sensitivity of the assay were reached at a relatively early time period, before it could be determined whether or not the elimination of L-PAM might actually be a triphasic phenomenon and parallel the elimination of total drug equivalents. There is some indication that this might be true, since small amounts of L-PAM were detected in the urine as long as 48 hours after dosing.

The rate of elimination of drug equivalents in the urine declined in a triphasic manner. When excretion of the parent compound was examined, only two phases of elimination were apparent; the most rapid phase was equivalent to the rapid elimination phase for total drug equivalents.

After iv dosing, the rate of fecal excretion declined in a first-order fashion through 5 days. Drug equivalents (in the form of ^{14}C) were detected for as long as 11 days after dosing, but the rate of elimination did not change beyond 5 days. The slow rate of fecal elimination may imply the presence of a rate-limiting process, related to metabolism, biliary excretion, covalent binding to macromolecules, or a combination of all three. The data are not sufficient to determine whether the process is zero order or very slow first order. A possibility is that L-PAM is metabolized extensively and that the constant rate of fecal elimination marks the presence of a different type of compound.

Experiments in mice and dogs (2) suggest that L-PAM is degraded in vivo to two products of hydrolysis and at least three metabolites. Experiments to isolate and identify those products are currently in progress. A method for isolating and analyzing drug equivalents excreted in the feces is not yet available; we do not know if the feces contain unaltered parent compound.

On the basis of total radioactivity, the relative distribution of drug equivalents described by Milner et al (6) and Cohn (7) for the rat was essentially the same as described here for the dog. Disappearance of drug equivalents from the various tissues roughly paralleled their elimination from serum. Drug equivalents were not sequestered in fatty tissue, nor did they cross the blood-brain barrier in great amounts. High concentrations of drug equivalents in the stomach after 30 minutes and 2 hours may have been due to reverse peristalsis in the gut, although no overt emetic actions were observed.

Excretion of L-PAM into the bile was immediate and rapid, and movement of drug equivalents into the contents of the ileum occurred within 4 hours. Although a high percentage (70%–80%) of the drug equivalents excreted in bile was parent compound, very little (0–2%) parent compound was found in the liver. It is not clear from the current studies why the excretion was so rapid (since L-PAM is a relatively small molecule for biliary excretion without conjugation) nor why L-PAM was so stable in the bile. Perhaps a macromolecular stabilizing effect similar to that reported for Tween 80, fibrinogen, and gamma-globulin (8) occurred. Approximately 10 ml of bile was harvested from the dog killed 30 minutes after dosing; 11% of the total dose administered was recovered in the bile at that time period. These data indicate that biliary excretion is a primary means of removing L-PAM and its products from the blood. High concentrations of drug equivalents were not found in the cecum or colon although sampling of these contents at a later time period would surely have yielded different results since drug equivalents were excreted in the feces.

L-PAM is as effective as 5-fluorouracil against certain gastrointestinal neoplasms and has fewer toxic effects; in a single case of gall bladder cancer, oral treatment with L-PAM yielded an objective remission of the disease (9). On the basis of the distribution studies reported here, it is not surprising that L-PAM was ineffective against gastric cancer.

The therapeutic implications for these data are that L-PAM should be given a trial against carcinoma of the gall bladder, carcinoma of the common bile duct, and perhaps carcinoma of the duodenum. The constraints are that hepatic or biliary dysfunction could seriously compromise the effectiveness of L-PAM.

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Some Observations on the Human Pharmacology of Methotrexate [1]

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SUMMARY

Plasma and urinary levels of methotrexate (MTX) have been measured enzymatically in 18 patients receiving doses of 5–1250 mg. When tritium-labeled MTX was administered, plasma levels measured by radioisotope counting were significantly higher than those measured enzymatically, the difference being accounted for by the presence of tritium label in plasma water as a result of drug metabolism. Renal clearance of MTX correlated well with glomerular filtration rate (GFR) and was consistently lower than the GFR, suggesting tubular reabsorption of the drug at the rate of urine flow studied. Plasma clearance measured by an infusion method was consistently greater than renal clearance, suggesting drug metabolism. Biliary MTX levels have been measured in three patients and are very much higher than plasma levels, suggesting a quantitatively important biliary recirculation of the drug. Plasma levels of MTX measured after 24 hours were not significantly different following iv or im administration, but were higher if an infusion was used.

[Cancer Treat Rep 61:1647–1656, 1977]

Methotrexate (MTX) is a widely used antitumor drug whose effectiveness is related to the extent to which the target enzyme, dihydrofolate reductase [EC 1.5.1.4], is inhibited (1,2). Its toxicity to normal and malignant cells is dependent on both the administered dose and the time of exposure to the drug (3,4), so the pharmacokinetics of MTX in man is an important determinant of its therapeutic effectiveness.

As would be expected for a drug possessing a wide spectrum of antitumor effectiveness, considerable attention has been devoted to its pharmacokinetics in man. However, there are discrepancies between the results of those workers who have studied the fate of tritiated MTX (5–8) and those who have adopted more specific methods of assay (9,10). Thus, for tritiated MTX, both Huffman et al (6) and Wan et al (7) describe a triphasic plasma decay with half-lives of approximately 0.75, 3.5, and 27 hours. In contrast, Tattersall et al (9) have measured plasma levels of MTX following high-dose administration both by the enzymatic method (11) and by radioimmunoassay (12) and report an exponential decay with a median half-life of 5.5 hours during the first 72 hours. Stoller et al (10), also using the enzymatic method, describe a biphasic decay with half-lives of approximately 2 and 10 hours. Further, Leme et al (8), studying the fate of tritiated MTX, have concluded that the third component of the plasma decay curve is not due to intact MTX. In cynomolgus and rhesus monkeys, Kimelberg et al (13) have shown extensive metabolism of tritiated MTX 4 hours after iv injection.

Freeman-Narrod et al have compared the circulating levels of MTX (using a fluorometric assay) achieved following its administration by various routes (14). It was found that eight of 17 patients receiving 15-mg/m^2 doses im had plasma levels > 40 ng/ml (approximately 10^{-7} M) at 24 hours, while none of six patients receiving iv doses had detectable levels at 24 hours.

Liegler et al (15) found a renal clearance of tritiated MTX somewhat in excess of the glomerular filtration rate (GFR) in human subjects. Bourke et al (16) found that the renal clearance increased as the plasma MTX level decreased in rhesus monkeys.

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In view of the discrepancies in the literature concerning the pharmacokinetics of MTX in man, we felt this problem needed further consideration. In this paper we have examined the possibility that disparate values of MTX half-lives may be the result of metabolism of the drug in man; in five patients the plasma levels of tritiated MTX were measured by both radioisotopic and enzymatic methods. Plasma half-lives of MTX given im or iv have also been measured and fitted to a mathematic model.[3] The renal handling of the drug and its biliary excretion have also been examined.

Because the clinical situation often demands that MTX be administered to patients with abnormal renal function, some patients with abnormal renal function were also studied.

PATIENTS AND METHODS

All patients received MTX as part of their normal treatment. Consequently, some patients received other drugs in addition to MTX. Details of the patients studied are shown in table 1. The possible effects which the other drugs may have had on the pharmacokinetics of MTX are discussed below. GFR was estimated by the ^{51}Cr EDTA method (17).

All infusions were maintained at a constant rate using a Tekemar pump. Blood samples were drawn from patients through an indwelling plastic cannula sited in the opposite arm to that in which the drug was given. In those patients who received bolus injections of MTX, blood samples were obtained at 5, 10, 30, and 60 minutes and at longer intervals (4–6 hours) thereafter. All blood samples were drawn into heparinized tubes and centrifuged within 1 minute of being taken. The plasma was stored at −20°C and was assayed for MTX within 4 weeks of collection. Urine was collected in plain glass bottles, the volume was measured, and aliquots were stored at − 20°C.

Bile samples were obtained at laparoscopy via a transhepatic aspiration of the gall bladder, and stored at –20°C until assay. In one patient, common bile duct bile was obtained directly from a "T" tube. Appropriate ethical permission and the informed consent of the patients were obtained for all procedures.

Samples were found to be stable at –20°C for at least 3 months, and no loss of drug was observed after three freeze-thaw cycles.

MTX for injection was purchased from Lederle Laboratories (Gosport, Hants, England). Tritiated MTX (labeled at the 3′, 5′, and 9 positions) was purchased from the Radiochemical Centre (Amersham, England) and purified by paper chromatography in 0.5% sodium carbonate. The band containing MTX was eluted in a 0.9% sodium chloride solution, mixed with the appropriate amount of unlabeled drug to achieve a specific activity of 0.8 curie/mol, and used within 24 hours. The radiochemical purity estimated by paper chromatography using (*a*) 0.5% sodium carbonate, and (*b*) *n*-butanol-pyridine-water (1:1:1) was > 99% in the preparation used for all patients except No. 1, when it measured 99% and 98.5% in the two systems, respectively.

Tritiated MTX levels in plasma were determined by scintillation counting of 1-ml aliquots of plasma in 10 ml of scintillation fluid and counted in an Intertechnique SL30 liquid scintillation spectrometer with a constant quench correction program. Urine samples (0.2-ml undiluted aliquots) were added to 0.3 ml of water and 10 ml of scintillation fluid and counted using a variable quench correction program. The scintillation fluid used was "PCS" supplied by Hopkin and Williams Ltd (Romford, Essex, England). A counting efficiency of 30% was achieved and there was no chemiluminescence from control plasma samples. In patient 1, 0.5-ml aliquots of plasma were solubilized in 0.5 ml of hyamine hydroxide and the scintillation fluid used had the following composition: toluene, 1925 ml; dioxan, 1925 ml; methanol, 1150 ml; butyl PBD, 35 g; and naphthalene, 400 g.

Enzymatic assays of MTX were performed using the method of Bertino and Fischer (11). Rat liver was used for the source of dihydrofolate reductase. It was possible to detect as little as 0.5 pmol of MTX with a standard error of ≤ 5% using duplicate assays.

Tritiated MTX levels in bile were measured after combustion of the freeze-dried sample in an Intertechnique Oxymat combuster. It was impossible to count bile samples directly owing to excessive quench.

Reagents were purchased from BDH Ltd (Poole, Dorset, England) or from Hopkin and Williams Ltd (Romford, Essex, England). AnalaR grades were used where available. NADPH was purchased from Boehringer Mannheim (Lewes, Sussex, England) and dihydrofolic acid from Sigma London Ltd.

In the following discussion of results all levels quoted are assayed enzymatically if not otherwise stated. The radioimmunoassay (12) was not used in this study.

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TABLE 1.—Details of patients studied

Patient No.	Diagnosis	Weight (kg)	Surface area (m^2)	GFR (ml/min)	Dose (mg) of MTX	Route	Bile specimens	Other drugs given
1	Carcinoma of bronchus	43	1.45	98	200 tritiated	iv	No	Vincristine, 1 mg; cyclophosphamide, 500 mg at 24 hrs; folinic acid, 9 mg at 24, 30, 36, and 42 hrs
2	Carcinoma of bronchus	43	1.40	61	200	iv	No	Cyclophosphamide, 1 g, + vincristine, 1.5 mg at 0 hr; adriamycin, 60 mg at 24 hrs
3	Carcinoma of bronchus	74	1.85	78	200	iv	No	Prednisolone, 40 mg at 4 hrs; folinic acid, 9 mg at 24, 30, 36, and 42 hrs
4	Carcinoma of bronchus	58	1.70	—	440 tritiated	iv	No	Folinic acid, 9 mg at 24, 30, 36, and 42 hrs
5	Acute lymphatic leukemia	74.5	1.67	127	1250	iv	No	L-Asparaginase at 24 hrs
6	Granulocytic sarcoma	5.7	1.43	58	15	iv	No	None
7	Aplastic anemia	15	0.65	25*	5	iv	No	None
8	Carcinoma of breast	82	1.99	—	50 tritiated	iv	Yes	None during period of study
9	Hodgkin's disease	71	1.90	—	50 tritiated	iv	Yes	None during period of study
10	Carcinoma of bladder	60	1.45	68†	440 tritiated	iv	No	Sodium citrate, 100 mg 3 times/day; acetazolamide, 500 mg at night; folinic acid at 7 hrs and at 6-hr intervals for 2 days
11	Carcinoma of bladder	71	1.80	78	200	im	No	Folinic acid, 9 mg at 6, 10, 18, and 24 hrs
12	Carcinoma of bladder	67	1.81	41	200	im	No	Folinic acid, 9 mg at 6, 10, 18, and 24 hrs
13	Carcinoma of bladder	81	1.97	89	200	im	No	Folinic acid, 9 mg at 6, 10, 18, and 24 hrs
14	Carcinoma of bladder	52	1.50	25	200	im	No	Folinic acid, 9 mg at 6 hrs and at 6-hr intervals for 3 days
15	Carcinoma of nasopharynx	60	1.63	165	200	iv infusion over 24 hrs	No	None during period of study
16	Carcinoma of larynx	61	1.67	82†	200	iv infusion over 24 hrs	No	None during period of study
17	Carcinoma of larynx	73	1.95	125	200	iv infusion over 24 hrs	No	None during period of study
18	Fibrosarcoma	83	2.08	70	100 daily	Intra-arterial infusion for 5 days	No	Continuous folinic acid

*Creatinine clearance—mean of 3 determinations.
†Creatinine clearance.

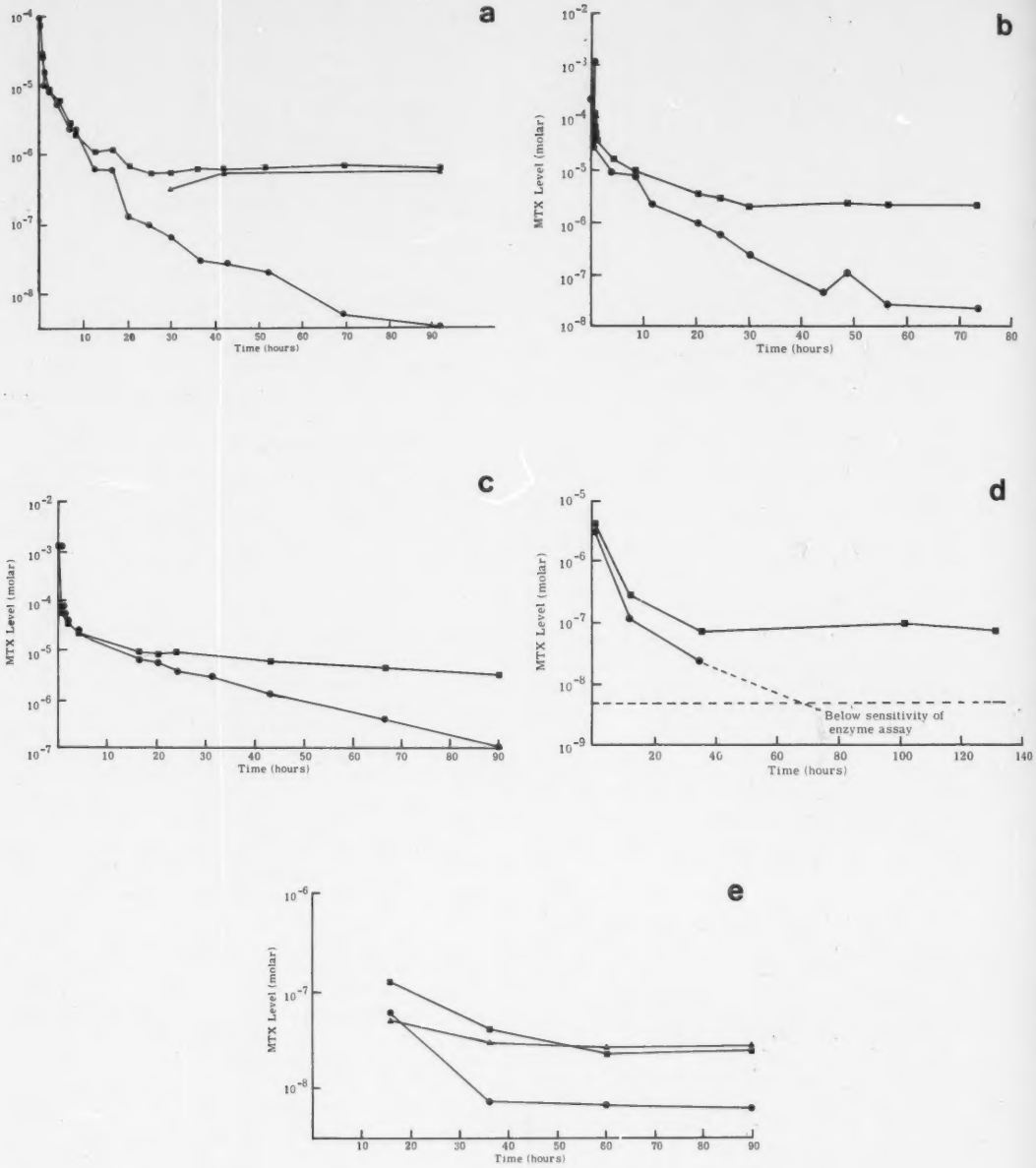

FIGURE 1.—Comparison of plasma levels of MTX in 5 patients assayed enzymatically (●——●) or radioisotopically (■——■) and of 3 H_2O levels in plasma (▲——▲): (*a*) patient 3; (*b*) patient 10; (*c*) patient 4; (*d*) patient 8; and (*e*) patient 9.

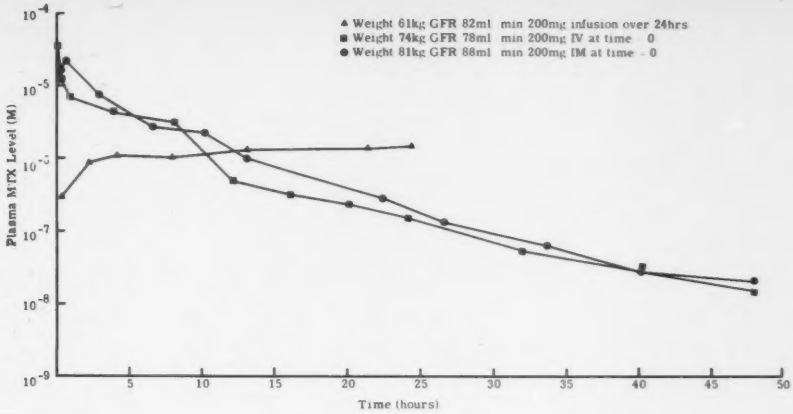
▲ Weight 61kg GFR 82ml min 200mg infusion over 24hrs
■ Weight 74kg GFR 78ml min 200mg IV at time 0
● Weight 81kg GFR 88ml min 200mg IM at time 0
Plasma MTX Level (M)
Time (hours)

FIGURE 2.—Effect of method of administration on plasma MTX levels in 3 patients.

RESULTS

Comparison of Results Obtained by Tritiated MTX Assay and Enzyme Inhibition Assay

Plasma levels of MTX in patients who had assays performed by both methods are shown in figure 1. It can be seen that for the first 12 hours after injection the agreement is good, but at later times there is a wide difference between the results of the two methods. At 90 hours the tritium counting method gives a result which is up to 100 times higher than the enzyme inhibition assay. In order to account for this difference, samples of plasma were freeze dried. The freeze-dried residue and the water were then counted separately. The results are shown in figure 1. In patient 1 the amount of radioactivity present as tritiated water was equal to the difference in the result of the two assays at 42 and 90 hours. However, at 30 hours, only 30% of the total radioactivity associated with the solute fraction was present as MTX (as measured by enzyme inhibition assay), suggesting the presence of a tritiated metabolite. In samples studied on each of the other patients from 24 hours on the difference between the two methods could be accounted for by tritiated water within the limits of the accuracy of the methods. To check the possibility that circulating dihydrofolate reductase might be present causing a falsely low result on the enzyme inhibition assay, samples of plasma were alkalinized to a pH of 8, boiled for 10 minutes, and then reassayed. The results were identical with the first assay. To check the possibility that tritium might be exchanging from the MTX with plasma protons, tritiated MTX was added to normal human plasma at a concentration of 4.5×10^{-6} M. Freeze drying of this plasma after 24 or 48 hours of incubation at 37°C showed no formation of tritiated water.

Plasma Concentrations of MTX Resulting From its Administration by Different Routes

Plasma concentrations of MTX after different routes of administration are shown in figure 2. The three patients were chosen to be reasonably closely matched in terms of weight and GFR. It can be seen that the plasma level was maintained at a higher level after the first 12 hours by a 24-hour infusion than by bolus administration of the same dose. There is little difference between the acute iv and im curves. In each case, the decay could be approximated by a biphasic curve. Details of MTX plasma levels in all patients studied are listed for three selected time points in table 2. The mean plasma levels at 24 hours in patients with comparable renal function were 1.2×10^{-7} M from a 200-mg iv dose (patients 1–3) and 1.9×10^{-7} M from a 200-mg im dose (patients 11–13).

In order to estimate half-lives, plasma decay curves from the patients receiving im or iv doses were fitted to a two-compartment pharmacokinetic model, using "FITME," a computer program designed for this purpose (*see* footnote 3). This is essentially an unweighted nonlinear regression of a double exponential function of the form $y = Ae^{-\alpha t} + Be^{-\beta t}$ using a least chi-squared fit. The two half-

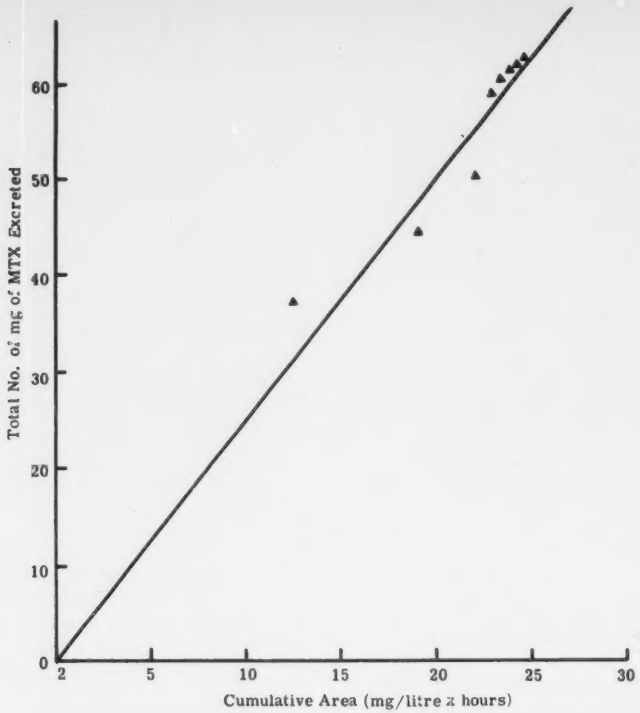

FIGURE 3.—Determination of the renal clearance of MTX from plasma levels and urine collections. Plasma level was $< 10^{-7}$ M for the last 3 points, but the gradient of the line does not change.

lives so derived were 33 minutes with a standard deviation of 7 minutes and 5.34 hours with a standard deviation of 1.83 hours. The figures for the second half-life cannot of course be considered representative for patients with normal renal function.

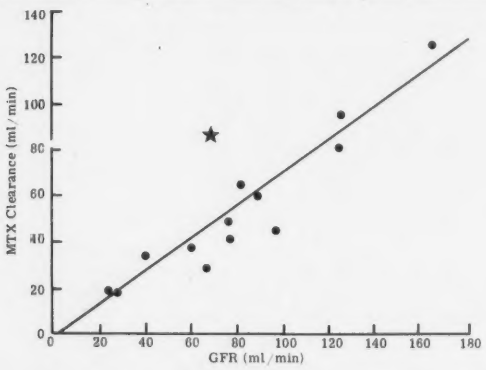

FIGURE 4.—Correlation between renal clearance of MTX (assayed enzymatically) and GFR. $y = 0.72–2$; $r = 0.89$; $P < 0.005$.

Renal Clearance of MTX

Renal clearance of MTX was obtained by plotting a graph of the cumulative area under the curve of plasma levels versus the total amount excreted to that time. An example is shown in figure 3. If the clearance of MTX is constant during the period of study the result will be a straight line whose slope is the clearance of MTX. If the clearance of MTX changes as a result of a change in the plasma level or the administration of another drug, the points will deviate from a straight line. In all of the cases studied, no consistent trend of the points was observed away from the best straight line, either with time or with the administration of the other drugs mentioned, including folinic acid.

There is good correlation between MTX clearance and GFR (as shown in fig 4), with the correlation coefficient (r) = 0.89. MTX clearance is in general lower than GFR, being about 0.72 × GFR. Patient 18 (marked by a star, fig 4) was unusual in that he had a higher MTX clearance than the GFR. This may have been due to the fact that he received a 5-

TABLE 2.—Measured plasma levels in individual patients at different times after administration of MTX

Patient No.	Route of administration	Plasma levels ($M \times 10^{-8}$) at—		
		6 hrs	24 hrs	48 hrs
1	iv	230	9.7	2.2
2	iv	260	11.0	0.73
3	iv	350	16.0	1.8
4	iv	867	56.0	10.3
5	iv	1258	20.9	3.64
6	iv	24	3.3	—
7	iv	26	2.0	—
10	iv	1659	352.0	93.0
11	im	410	10.0	—
12	im	500	26.0	2.2
13	im	400	22.0	2.0
14	im	1500	240.0	15.0
		Plateau level of infusion		
15	iv infusion	140		
16	iv infusion	140		
17	iv infusion	150		
18	iv infusion	88		

TABLE 3.—Plasma and urinary clearances of MTX measured in patients having iv infusions showing significant clearance by the nonrenal route

Patient No.	Clearances (ml/min)		
	Plasma	Urine	Difference (nonurinary clearance)
10	166	125	41
11	171	64	107
12	159	81	78
13	167	85	82

day intra-arterial perfusion of MTX diluted in large volumes of normal saline and therefore maintained a high urine output. If this patient is omitted from the analysis, the correlation is better, $r = 0.95$. MTX recovery in the urine averaged 54%, with a range of 31%–80% in the first 48 hours. In one patient who was followed for 90 hours, only a further 0.5% of the administered dose was recovered. The highest urinary recovery (80%) was found in the patient receiving the highest dose of MTX (No. 5).

Plasma Clearances of MTX

These were determined in four patients (Nos. 15–18) having iv infusions. Plasma samples were taken at 4–6-hour intervals, and in every case the plasma level of MTX reached a plateau during the time of the infusion. The plasma clearance (C) of MTX was calculated from the plateau level of the drug (L) and the rate of infusion (R) by equating the rate of infusion with the rate of elimination:

$$\text{ie, } C = R/L.$$

The results are shown in table 3. In every case, the plasma clearance of the drug was higher than the urinary clearance. This could be due to either excretion of the drug by some route other than the urine, metabolism to an inactive form, or binding to sites which did not become saturated.

Biliary Levels of MTX

MTX levels in gall bladder bile were assayed in two patients who received 50-mg iv doses of tritiated MTX. In both patients the levels assayed either radioisotopically or enzymatically were identical, and showed a dramatic concentration of the drug in the bile, the level in one patient being 10,000

TABLE 4.—Biliary levels of MTX*

Patient No.	Time (hrs) of sample	Bile MTX level (M × 10^{-4})		Plasma MTX level (M), enzymatic
		Radioisotope	Enzymatic	
8	12	9.33	9.65	1.23×10^{-7}
9	16	3.18	2.86	6.21×10^{-8}

*Shown for gall bladder bile sampled at the times indicated after a 50-mg iv dose. Plasma decay curves for these patients are shown in fig 1d and e.

TABLE 5.—MTX levels in common bile duct bile following a 50-mg iv dose

Bile collection period (hrs)	MTX level (M)
0–3	1.5×10^{-5}
3–6	1.2×10^{-5}
6–9	5.9×10^{-6}
9–12	9.6×10^{-6}

times higher than the simultaneous plasma level, and in the other patient, 2500 times higher. These results are summarized in table 4.

Biliary levels of MTX were also determined in one patient who had a "T tube" sited in the common bile duct. He received 50 mg of MTX iv. The levels of MTX assayed in four serial bile collections are shown in table 5.

DISCUSSION

These data from patients receiving tritiated MTX suggest that the drug undergoes conversion to a metabolite. The presence of much of the injected MTX tritium as water after 30 hours could be due either to metabolism of the drug resulting in the formation of tritiated water, or to exchange of tritium with protons in the body. The latter possibility has been discounted by the results discussed above.

The presence of metabolites of MTX in man has been suggested by Huffman et al (6) and 7-hydroxymethotrexate has been described previously as a human metabolite (18). A number of other metabolites have been described in the rat (19,20) of which 4-amino-4-deoxy-N^{10}-methyl pteroic acid occurs in the largest quantities. Metabolism to either 7-hydroxymethotrexate or 4-amino-4-deoxy-N^{10}-methyl pteroic acid will not result in the formation of tritiated water since neither process involves the 3′, 5′, or 9 positions on which the tritium of radiolabeled MTX was located. However, a number of 2,4-diamino pteridine metabolites of MTX with various substituents at the 6 position (methyl, hydroxymethyl, aldehyde, and carboxyl groups) were also described in rats (19,20). The formation of one of these in man could result in the appearance of tritiated water, since in such cases the C9-N10 bond is involved.

In patients studied here, numerous irregularities were seen in the plasma decay curves. A curve in such a patient (No. 3) in whom this effect was particularly marked is shown in figure 2. The plasma levels tended to be higher after meals than before, suggesting biliary excretion and recirculation of the drug. Creaven et al (21) reported very little biliary excretion of tritium-labeled MTX in one patient who had a biliary fistula. However, in the patients studied by us, who all received 50 mg of MTX iv, levels of between 10^{-3} and 10^{-4} M MTX were observed in gall bladder bile, and 10^{-5} M levels were seen in bile from the common bile duct. These levels are all vastly higher than the plasma levels measured over the same period, indicating a considerable concentration of the drug in the bile. Indeed, the first patient shown in table 5 had approximately 20 ml of bile in the gall bladder which therefore contained 10 mg of MTX or 20% of the administered dose. The extensive biliary recirculation of the drug may explain the fluctuations which we observed in the plasma decay curves.

The plasma levels measured in this series of patients could in every case be approximated by a biphasic curve during the first 24 hours for which they were followed, suggesting a two-compartment distribution. The presence of the third half-life observed in those studies using tritium-labeled MTX, is probably at least in part due to the presence of tritiated water. However, in the patients followed for longer periods (figs 1 and 5) there is not a clear terminal half-life, suggesting a more complex system such as that described by Zaharko et al (22).

The renal clearances of MTX observed here were considerably lower than those reported either by Liegler et al (15) or by Bourke et al (16). However, in both of these studies a high urine flow was maintained throughout. No attempt to do this was made in our patients and the normal rate of urine

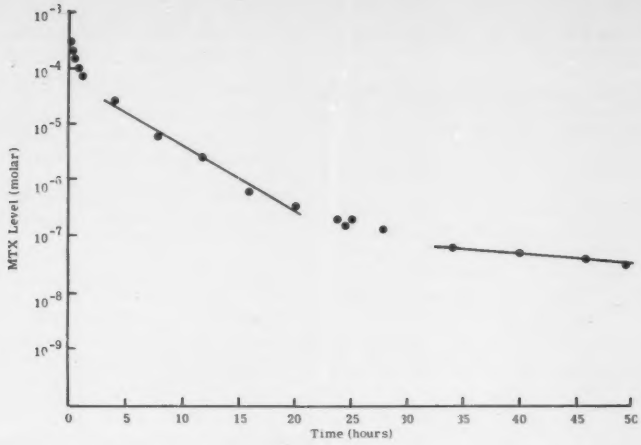

FIGURE 5.—Plasma decay curve of a patient receiving 1250 mg of MTX iv (assayed enzymatically), showing a 2nd half-life of 2.82 hrs and a clear 3rd phase with a half-life of approximately 16 hrs.

flow was about 80 ml/hour. It is possible that a lower urine flow rate permits greater reabsorption of MTX from the renal tubules or collecting ducts, thus reducing the overall clearance. Reabsorption of MTX probably occurred in all except one of our patients (No. 18) since in every case MTX clearance was less than GFR.

The fate of MTX cleared from the plasma by the nonrenal route is not known at present. However, fecal excretion has been reported to be relatively unimportant using tritiated MTX (6). Binding of the drug to nonsaturable sites seems unlikely. Based upon the observation that while assaying MTX it is necessary to add about 5 ng of MTX to significantly inhibit dihydrofolate reductase from an aliquot prepared from 10 mg of liver, it can be calculated that the amount of dihydrofolate reductase normally present in liver would be expected to bind only about 0.5 mg/kg of the drug. Patient 18 received a total of 500 mg over 5 days with no tendency for the plasma level to increase, which would be expected if binding sites became saturated.

A likely explanation is that there is extensive metabolism of the drug accounting for up to 50% of the administered dose as shown by the consistently incomplete recovery in the urine. This hypothesis is also consistent with the observations of Kimelberg et al (13) in monkeys.

The extracellular concentration of MTX necessary for significant inhibition of DNA synthesis is known for various murine tissues (23) where it is reported to be about 10^{-8} M. Preliminary unpublished work from this laboratory indicates that a level of 10^{-6} M may be necessary for human breast tumors.[4] Clinical studies by Jaffe et al (24,25) suggest that even higher levels may be necessary for osteogenic sarcoma. The data presented here suggest that a plasma level $> 10^{-6}$ M will not usually be maintained for a period of 24 hours in an adult if a single bolus of 200 mg is given. However, a level of 10^{-6} M may be maintained for 24 hours with an infusion of about 200 mg. Thus, it may be that doses of this order may be giving the patient a less than optimal exposure to the drug if given as a single injection.

In view of the dependence of the clinical effectiveness of the drug on its pharmacokinetics, it is obviously desirable to be able to predict plasma levels using a mathematic model. A simple linear two-compartment model has been fitted to the data obtained here by the enzymatic assay and is to be published separately (*see* footnote 3).

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A Microbiologic Disc Assay for 5-Methyltetrahydrofolate in the Presence of Methotrexate [1,2]

Bipin M. Mehta* and Dorris J. Hutchison [3,4]

SUMMARY

High-dose methotrexate-citrovorum factor rescue regimens are used extensively for the treatment of human malignancies. Since the rescue agent citrovorum factor is rapidly converted to 5-methyltetrahydrofolate, it is desirable to follow the distribution kinetics of both of these coenzymes to assess the selective therapeutic effectiveness of the regimen. The assay methods using methotrexate-resistant strains of *Lactobacillus casei* ATCC 7469 (LC/A), *Streptococcus faecium* var. *durans* ATCC 8043 (SF/A), and *Pediococcus cerevisiae* ATCC 8081 (PC/A) have been developed to determine the concentrations of 5-methyltetrahydrofolate and other folates in the presence of methotrexate. Since LC/A determines the total folates, SF/A determines the total folates except 5-methyltetrahydrofolate, and PC/A by our procedure determines only the most stable form of reduced folates, citrovorum factor, the quantitation of both 5-methyltetrahydrofolate and citrovorum factor can very easily be achieved by differential assays. This report describes the determination of as low as 10 ng/ml of 5-methyltetrahydrofolate in the presence of as high as 500 μg/ml of methotrexate. The assay method described here is currently used in studying the pharmacokinetics of 5-methyltetrahydrofolate in patients with meningeal neoplasms.

[Cancer Treat Rep 61:1657–1663, 1977]

Methotrexate, by inhibition of dihydrofolate reductase, depletes the reduced folates which are essential co-factors not only in thymidylate synthesis but also in purine and protein synthesis. Citrovorum factor, the most stable form of the reduced folates (1,2), is used therapeutically to selectively reverse the effect of methotrexate (3–6) and thereby rescue normal cells. Although citrovorum factor is used for the recovery of rapidly proliferating normal cells, it is desirable to follow the distribution kinetics of both methotrexate and citrovorum factor in cancer patients with respect to their therapeutic response and to correlate doses of methotrexate and citrovorum factor with therapeutic efficacy and toxicity. Both methotrexate and citrovorum factor concentrations are determined routinely in our laboratory (7,8).

Nixon and Bertino (9) reported rapid conversion of citrovorum factor to 5-methyltetrahydrofolate following oral administration. Since 5-methyltetrahydrofolate is the major component of the naturally occurring serum folates (10) and is readily transported into and utilized by tissues (11), the determination of serum 5-methyltetrahydrofolate is another appropriate parameter to compare with serum methotrexate levels.

Herbert et al (10) demonstrated that 5-methyltetrahydrofolate is the major folate component of the human serum; this was further substantiated by Noronha and Aboobaker (12) and by Bird et al (13). Herbert et al (10) further demonstrated that the folic acid activity in human serum for *Lactobacillus casei* is not made available to *Streptococcus faecium*

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var. *durans* and *Leuconostoc citrovorum* (presently known as *Pediococcus cerevisiae* [14]) by the action of hog kidney or chicken pancreas conjugase, and that 5-methyltetrahydrofolate has marked folic acid activity for *L. casei* but relatively little for *S. faecium* var. *durans* or *P. cerevisiae*. Since *S. faecium* var. *durans* responds to all forms of folates except 5-methyltetrahydrofolate, a differential assay system, if developed, could determine the amount of 5-methyltetrahydrofolate in the human serum. These assumptions led us to isolate a methotrexate-resistant strain of *L. casei* (LC/A) in order to determine 5-methyltetrahydrofolate in the presence of high concentrations of methotrexate. A methotrexate-resistant strain of *S. faecium* var. *durans* (SF/A), already a part of our culture collection (15), is used to determine the total folates, except 5-methyltetrahydrofolate, in human serum. Citrovorum factor in the presence of methotrexate is routinely determined using a methotrexate-resistant strain of *P. cerevisiae* (PC/A) (16). With the use of LC/A, total reduced folates in the human serum can be determined and then through the difference in activities for LC/A and SF/A, one can very easily calculate the serum levels of 5-methyltetrahydrofolate.

This report describes a differential microbiologic disc-assay procedure, using LC/A, SF/A, and PC/A to determine the concentrations of 5-methyltetrahydrofolate and citrovorum factor in the presence of relatively high concentrations of methotrexate.

METHODS

Organisms[5]

LC/A was isolated by a procedure similar to one described by Hutchison and Burchenal (16) for the isolation of PC/A. The development of resistance in LC/A as shown in figure 1 followed a pattern similar to that obtained for PC/A (16). Through 22 transfers covering a period of 120 days, a culture with about a 400,000-fold increase in resistance to methotrexate was selected. The culture LC/A was then arbitrarily maintained in the *L. casei* medium of Flynn et al (17) containing methotrexate (50 μg/ml) and folic acid (1 ng/ml).

SF/A is a part of our culture collection. The isolation and maintenance of this mutant have been described by Hutchison (18).

PC/A, isolated by Hutchison and Burchenal (16), is used for citrovorum factor assay as described earlier (7).

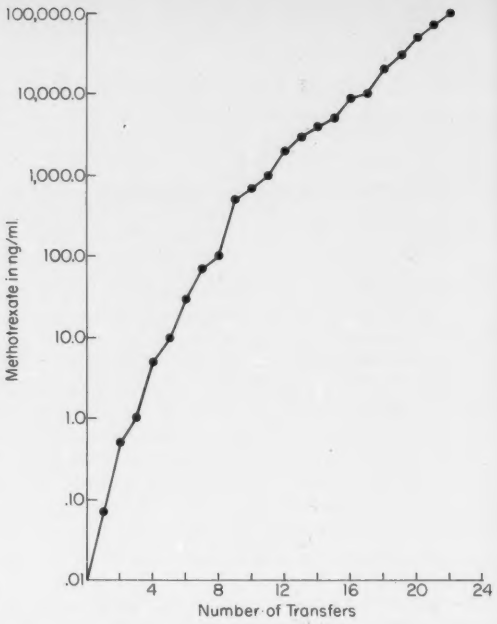

FIGURE 1.—Development of resistance to methotrexate by *L. casei* ATCC 7469.

Growth Requirements

The 5-methyltetrahydrofolate, folic acid, and citrovorum factor requirements for the three methotrexate-resistant strains, LC/A, SF/A, and PC/A, were compared with those of the parent methotrexate-sensitive strains, LC/0, SF/0, and PC/0, respectively, by the microbiologic vitamin-assay technique as described by Hutchison (18). Varying concentrations of 5-methyltetrahydrofolate (Sigma Chemical Co, St. Louis, Mo) and folic acid and citrovorum factor (both from Lederle Laboratories, Division of American Cyanamid, Pearl River, NY) between 0.01 and 100 ng/ml were used in *L. casei* medium (17) for LC/0 and LC/A, in *S. faecalis* medium (17) for SF/0 and SF/A, and in *P. cerevisiae* medium (19) for PC/0 and PC/A. The concentrations of 5-methyltetrahydrofolate, folic acid, and citrovorum factor required for half-maximum growth of the methotrexate-sensitive and methotrexate-resistant strains of *L. casei*, *S. faecium* var. *durans*, and *P. cerevisiae* are given in table 1. The commercially available stable DL-forms

[5]Organisms used in this study may be obtained free of charge from our laboratory in addition to any information about the use of these organisms.

TABLE 1.—5-Methyltetrahydrofolate, folic acid, and citrovorum factor requirements for methotrexate-sensitive and methotrexate-resistant *L. casei*, *S. faecium* var. *durans*, and *P. cerevisiae**

Strains	ng/ml for half-maximum growth		
	5-Methyl-tetrahydro-folate	Folic acid	Citrovorum factor
LC/0	0.15	0.15	0.15
LC/A	0.15	0.15	0.15
SF/0	15.0	0.15	0.30
SF/A	15.0	0.15	0.30
PC/0	30.0	20.0	0.03
PC/A	50.0	50.0	0.05

*See text for experimental details.

of 5-methyltetrahydrofolate and citrovorum factor are only half as active as the naturally occurring L-forms to which the organisms respond; hence, this difference has been taken into consideration in all of our calculations.

Methotrexate Sensitivity

Inhibition of growth of LC/A, SF/A, and PC/A was compared with that of the respective sensitive strains, LC/0, SF/0, and PC/0, using the tube-assay technique similar to the one we described for cytosine arabinoside (20). Growth-inhibition assay for methotrexate (Lederle Laboratories, Division of American Cyanamid, Pearl River, NY) was carried out in the *L. casei* medium (17) containing 2.5 ng/ml of 5-methyltetrahydrofolate for LC/0 and LC/A, in *S. faecalis* medium (17) containing 1.0 ng/ml of folic acid for SF/0 and SF/A, and in *P. cerevisiae* medium (19) containing 1 ng/ml of citrovorum factor for PC/0 and PC/A. The concentrations of methotrexate required for 50% inhibition of growth are given in table 2.

Disc Assays of 5-Methyltetrahydrofolate and Folic Acid

The disc assay for citrovorum factor has been described by us (7) and was followed for the determination of citrovorum factor. Since both the 5-methyltetrahydrofolate and folic acid assays rely on the measurement of zones of growth similar to those obtained with the citrovorum factor (7), the assay media (17) did not contain 5-methyltetrahydrofolate or folic acid usually required for the growth of the respective organisms. The *L. casei* assay medium was further supplemented with 1 mg/ml of ascorbic acid and its pH was adjusted to 6.8 prior to sterilization. The assay organisms, LC/A and SF/A, were grown overnight for 18 hours at 37°C in their respective liquid-assay media (17); both were supplemented with 1 ng/ml of folic acid. Both cultures were washed three times and resuspended in saline (0.85%) to an optical density of 0.2 at 600 nm (approximately 4×10^7 cells/ml) as measured in a Bausch and Lomb "spectronic 20" spectrophotometer; 5.0 ml of each of the suspensions was used as the inoculum for 200 ml of the respective assay media which also contained 1.5% agar. Flat-bottom glass Petri plates (100 × 15 mm) containing 10 ml each of the seeded agar medium were prepared and stored in the cold at 4°C for at least 3–4 hours prior to their use for the assays. For the 5-methyltetrahydrofolate standard curve (LC/A), the standard solutions were made in 0.05 M phosphate buffer, pH 7.5, containing 1 mg/ml of ascorbic acid, at concentrations of 12.5, 25, 75, and 125 ng/ml. For the folic acid standard curve (SF/A) the solutions were made in saline (0.85% at concentrations of 10, 50, 100, and 500 ng/ml). Paper discs (Schleicher and Schuell No. 740-E), 6.35 mm in diameter, were used for both of these assays. Five discs for each concentration of 5-methyltetrahydrofolate and folic acid were saturated with 0.02 ml of

TABLE 2.—Growth inhibition by methotrexate of methotrexate-sensitive and methotrexate-resistant strains of *L. casei*, *S. faecium* var. *durans*, and *P. cerevisiae**

Strains	Methotrexate—†	
	Sensitive	Resistant
L. casei	0.01	500,000.0
S. faecium var. *durans*	0.05	15,000.0
P. cerevisiae	0.015	25,000.0

*See text for growth medium and conditions.
†ng/ml for 50% growth inhibition.

the respective standard solutions and were dried for at least 1½ hours on a grooved stainless steel plate at 37°C. The discs were placed on agar plates and incubated at 37°C for 18 hours for folic acid assay and for 24 hours (or more, if required) for 5-methyltetrahydrofolate assay. The diameters, in millimeters of the growth zones (fig 2), were measured manually or with the Fisher Zone Reader. Standard curves were plotted as the log concentrations versus the average zone size (diameter in millimeters). No differences were observed in the growth response to 5-methyltetrahydrofolate and folic acid in the presence of from 0 to 5 μg/ml of methotrexate (fig 3). Higher concentrations of methotrexate carried substantial amounts of folic acid as an impurity, and therefore, larger growth zones

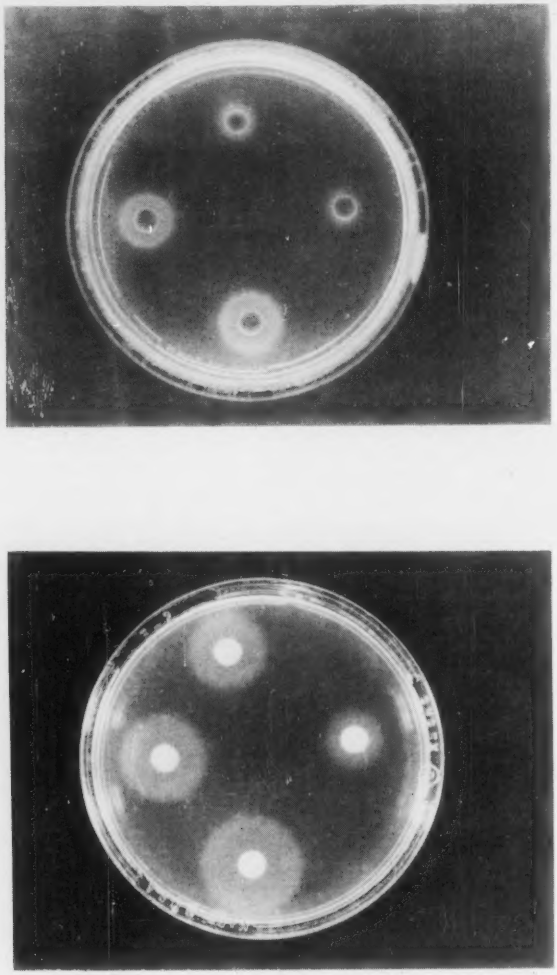

FIGURE 2.—(A) Growth of methotrexate-resistant L. *casei* with 5-methyltetrahydrofolate zones of growth with standard solutions of 5-methyltetrahydrofolate containing 12.5, 25, 75, and 125 ng/ml in 0.05 M phosphate buffer, pH 7.5, with 1 ng/ml of ascorbic acid; 0.02 ml of each solution applied to each of the respective discs (6.35-mm diameter). (B) Growth of methotrexate-resistant *S. faecium* var. *durans* ATCC 8043 with folic acid. Zones of growth with standard solutions of folic acid containing 10, 50, 100, and 500 ng/ml in 0.85% saline; 0.02 ml of each solution applied to each of the respective discs (6.35-mm diameter).

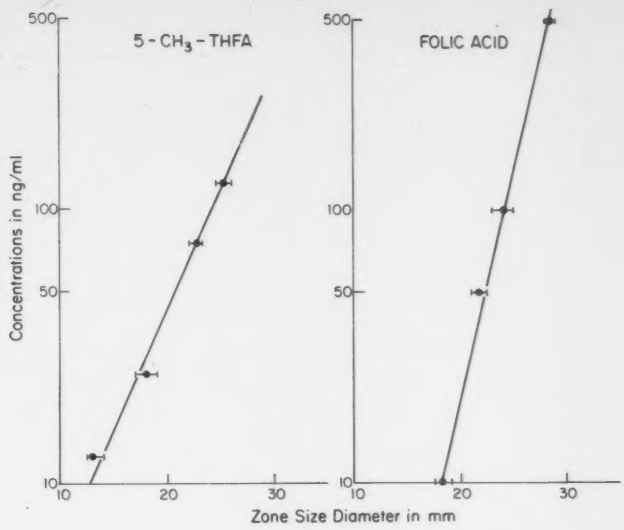

FIGURE 3.—Effect of methotrexate on 5-methyltetrahydrofolate and folic acid assay with methotrexate-resistant *L. casei* and *S faecium* var. *durans*, respectively. Prior to assay, methotrexate at 0, 5, 50, 500, and 5000 ng/ml was added to each of the standard solutions. Variations due to the presence of methotrexate are represented by bars.

may be obtained. The folic acid content of a sample can be determined by the SF/A assay method, and it is possible to eliminate the interference by difference. The recovery of both 5-methyltetrahydrofolate and folic acid from human serum and cerebrospinal fluid (CSF) was 98%–100%.

A comparison of the quantitation of the folate was made using known concentrations of 5-methyltetrahydrofolate (250 and 1250 ng/ml), folic acid (500 and 2500 ng/ml), and citrovorum factor (250 and 1250 ng/ml) (table 3) with the disc-assay methods using LC/A, SF/A, and PC/A and standard solutions of 5-methyltetrahydrofolate, folic acid, and citrovorum factor, respectively.

Determination of 5-methyltetrahydrofolate, folic acid, and citrovorum factor in mixtures was carried out using the assay techniques described here. Since LC/A determines the total reduced folate activity including 5-methyltetrahydrofolate and SF/A determines the total reduced folates except 5-methyltetrahydrofolate, the difference in the LC/A and SF/A activities should give us the total 5-methyltetrahydrofolate activity. Similarly, PC/A determines only the citrovorum factor, and the difference between the SF/A and PC/A activities should give us the activity equal to folic acid. These differential calculations have been applied in the determination of the amounts of 5-methyltetrahydrofolate, folic acid, and citrovorum factor present in the mixtures given in table 4.

RESULTS AND DISCUSSION

The 5-methyltetrahydrofolate requirement of LC/A and LC/0, the folic acid requirements of SF/A and SF/0, and the citrovorum factor requirements of PC/A and PC/0 are relatively unchanged (table 1). However, in the absence of sodium ascorbate, the 5-methyltetrahydrofolate requirement is about three times greater than in the presence of ascorbate. The folic acid and citrovorum factor requirements of SF/A and SF/0, and PC/A and PC/0, respectively, remain unaltered in the presence and absence of ascorbate. In the absence of ascorbate, however, the strain LC/A, when grown with 5-methyltetrahydrofolate, was less resistant to methotrexate (50% inhibition level approximately 150 μg/ml), whereas ascorbate had no effect on the methotrexate resistance of SF/A and PC/A.

The quantitation of 5-methyltetrahydrofolate, folic acid, and citrovorum factor by LC/A, SF/A, and PC/A as presented in table 3, shows clearly that LC/A utilizes all three compounds, whereas SF/A responds to folic acid and citrovorum factor but not to 5-methyltetrahydrofolate, and PC/A responds to citrovorum factor only at low concentra-

TABLE 3.—5-Methyltetrahydrofolate, folic acid, and citrovorum factor activity as measured by disc assay using LC/A, SF/A, and PC/A

Compound	Concentration (ng/ml)	ng/ml		
		LC/A activity as 5-methyltetrahydrofolate	SF/A activity as folic acid	PC/A activity as citrovorum factor
5-Methyltetrahydrofolate	250	227.3	—	—
5-Methyltetrahydrofolate	1250	1066.7	—	—
Folic acid	500	395.0	382.5	—
Folic acid	2500	2850.0	2350.0	—
Citrovorum factor	250	147.5	162.5	241.6
Citrovorum factor	1250	1150.0	900.0	1066.7

TABLE 4.—Use of the disc-assay methods in determining the concentrations of 5-methyltetrahydrofolate, folic acid, and citrovorum factor in mixtures

Compound	Concentration (ng/ml)	Total activity (ng/ml) of—			
		LC/A	SF/A	PC/A	Amount assayed*
Mixture I					
5-Methyltetrahydrofolate	1250.0	2000.0			1190.0
Folic acid	500.0		810.0		545.0
Citrovorum factor	250.0			265.0	265.0
Mixture II					
5-Methyltetrahydrofolate	250.0	2000.0			225.0
Folic acid	500.0		1775.0		525.0
Citrovorum factor	1250.0			1250.0	1250.0
Mixture III					
5-Methyltetrahydrofolate	250.0	2600.0			200.0
Folic acid	2500.0		2400.0		2135.0
Citrovorum factor	250.0			265.0	265.0

*Amount of 5-methyltetrahydrofolate was calculated by subtracting the total SF/A activity from the total LC/A activity of the mixture and the amount of folic acid was calculated by subtracting the total PC/A activity from the total SF/A activity of the mixture. Amount of citrovorum factor was the total PC/A activity of the mixture as such.

tions. The values given in table 3 are the mean values from three separate determinations and are within 84.14% ± 15.03% (SD).

Since PC/A determines the citrovorum factor alone, the value of citrovorum factor obtained by the PC/A assay method (7) would be the absolute value in a mixture (table 4). LC/A determines the total reduced folates whereas SF/A determines total reduced folates except 5-methyltetrahydrofolate. Thus, by subtracting the reduced folate activity determined by SF/A from the total reduced folate activity determined by LC/A, one obtains the residual 5-methyltetrahydrofolate activity. Also, subtracting PC/A activity from SF/A activity would give all folate activities except 5-methyltetrahydrofolate and citrovorum factor. The values obtained for each of the compounds (table 4) by this procedure were within 97.4% ± 10.3% (SD).

Waters and Mollins (21) suggested that the material in human serum with activity for *L. casei* is made up of a stable and a labile component, and the amount of the labile component varies; however, in normal subjects about 94% of the total serum *L. casei* activity was attributed to the labile component. Subsequently, Herbert et al (10), confirming the findings of Donaldson and Keresztesy (22), suggested that serum folate is identical with a monoglutamate, N^5-methyltetrahydrofolate, which also supports growth of *L. casei* alone. The *L. casei* activity of the labile component is lost during autoclaving or storage at –20°C. According to Waters and Mollins (21) the loss during autoclaving can be prevented by using adequate amounts of ascorbic acid in the phosphate buffer used to dilute the serum for assay and also by adding ascorbic acid to serum that is to be stored. Moreover, Waters and

Mollins (21) suggested that the activity lost during storage of serum not protected by ascorbic acid could be restored, for periods up to 3 months, by adding ascorbic acid to the serum prior to determinations. We have been able to confirm this report in our study with human serum as well as with CSF (unpublished data). Since the blood and CSF samples are obtained at the hospital bed and the laboratory usually receives the frozen samples, it has been customary in our laboratory to add Na-ascorbate (1 mg/ml), pH 6.8, before assaying the samples, even though our assay procedure does not require autoclaving of the samples prior to assay. Ascorbic acid at concentrations of > 1 mg/ml has been found to stimulate growth of the assay organisms to some extent. The recovery of 5-methyltetrahydrofolate from human serum and CSF was 98%–100%.

The disc-assay procedure described here has been adopted in our preliminary studies of pharmacokinetics in patients with meningeal leukemia receiving a high-dose methotrexate-citrovorum factor rescue regimen. Results from the use of this assay procedure in our opinion should prove to be a distinct asset for the determination of the effectiveness of the high-dose methotrexate-citrovorum factor rescue regimen. The knowledge of citrovorum factor and 5-methyltetrahydrofolate levels at various times during the treatment protocol may provide us with a better understanding of the role of citrovorum factor or its products as rescue agents.

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Pharmacokinetics of High-Dose Methotrexate With Citrovorum Factor Rescue [1,2]

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SUMMARY

The methotrexate (MTX)-plasma concentrations of 172 high-dose infusions over the range of 50–200 mg/kg were measured over the 72-hour period following the beginning of infusion. Pharmacokinetic analysis shows that a biexponential function adequately describes the plasma decay for all doses. The distribution of plasma clearances over the patient population at a given dose has been characterized by a biexponential clearance function and associated time-dependent variance. It is found that when each of the plasma clearance functions are scaled by their respective dose, the 1 SD bands about the resulting unit dose curves overlap throughout their time ranges and are therefore insignificantly different from one another. Thus, the plasma clearance over the 50–200-mg/kg range may be represented by a single dose-scalable biexponential model with half-lives of 1.8 ± 0.1 and 8.4 ± 0.5 hours. For a given maximum allowable plasma-MTX level (eg, 10^{-5} M at 24 hours), the variance of the clearance distribution is shown to predict the expected fraction of patients who will require intensified rescue. Urinary clearance has been determined at 104 ± 8 ml/minute over the dose range of 50–300 mg/kg and only 60% of the MTX was excreted in the urine by 72 hours.

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High-dose methotrexate (HDMTX) therapy followed by citrovorum factor (CF) rescue offers a new and potentially more efficacious treatment for sarcomas, lymphomas, and selected advanced carcinomas (1–6). The risk of severe toxicity from such therapy is now minimized by enhancing the plasma-MTX clearance with a forced alkaline diuresis (7,8). However, if the plasma-MTX concentration remains $> 10^{-5}$ M at 24 hours or 5×10^{-7} M at 48 hours, significant toxicity is likely to occur (9,10). If inadequate plasma-MTX clearance is identified within 24–36 hours following therapy, intervention by intensification of the CF rescue can reduce complications and in some cases may be life-saving (11,12). Severe toxicity may be avoided if the dose of CF administered achieves a plasma concentration estimated to exceed that of the plasma-MTX concentration (10,12).

In order to predict the clearance of MTX from the blood in an individual patient, it is important to quantify the pharmacokinetic parameters of MTX which may be influenced by dose and rates of infusion. A variety of high-dose protocols have been evaluated in clinical trials where doses ranged from 50 to > 500 mg/kg of body weight (13). It is clinically useful to characterize the pharmacokinetics of MTX at the differing doses to determine the impact of dose level on drug pharmacology and the frequency and severity of clinical toxicity. Such a reliable pharmacokinetic description permits clinical trials of HDMTX antitumor efficacy with improved assurance that severe toxicity can be predictably controlled. Additional dose-dependent pharmacokinetic

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data are needed for the development of models for tumor and normal tissue distribution of MTX leading to improved clinical protocol design.

In order to facilitate the use of HDMTX in clinical trial, this report presents an analysis of clinical pharmacologic data obtained from 82 patients during 172 courses of high-dose therapy. Characteristics of plasma-MTX pharmacokinetics at high dose levels following 4-hour infusions have been defined. Urinary excretion data for HDMTX were also studied.

Because of the large amount of data obtained in this study, it is possible to perform a somewhat more elaborate statistical analysis than is customary for clinical pharmacokinetic measurements. This analysis is unique in that, for a particular point in time, it derives the variance of an individually measured plasma-MTX concentration from the mean expected value characteristic of the patient population under study. This variance is shown below in the form of a time-dependent band which is ± 1 SD about the mean plasma-decay curve.

After scaling the MTX-plasma concentrations by dose and computing the SD for each dose level as a function of time, it is found that error bands, for all doses, overlap throughout the period. From this, we infer that a single scalable serum pharmacokinetic model can be used to describe the plasma clearance for all doses between 50 and 200 mg/kg.

Another use of the present data and analysis, both here and in future clinical investigations involving this protocol, is to assess the probability of a single patient requiring intensified rescue using the variance function described below. Because the variance describes a distribution of patient log-MTX concentrations at a particular time (eg, 24 hours), that portion of the distribution in excess of a given acceptable plasma level (eg, 10^{-5} M) predicts the fraction of patients who will require intensified rescue. Such a prediction may be helpful in anticipating the expected level of rescue if intensified rescue threshold levels are increased above the 10^{-5} M value employed in the present protocol. Such an increase could occur, for example, if a greater fraction of patients were brought closer to the drug-induced toxicity threshold value as part of a therapeutic scheme designed to increase antitumor effect for the maximum number of patients.

MATERIALS AND METHODS

Over 100 patients (4–68 years of age) received 239 high-dose infusions of MTX followed by CF rescue for therapy of various sarcomas and advanced carcinomas. Therapy was given for existing clinical disease or in an adjuvant setting where metastatic spread was expected but not clinically demonstrable.

The protocol has been described in detail by Isacoff et al (12) but is summarized as follows. All patients are hospitalized while receiving therapy. Creatinine clearance must be > 60 ml/minute prior to treatment. Balanced salt solutions in 5% dextrose were administered iv at the rate of 3 liters/m^2/day beginning 12 hours prior to the MTX infusion. Diamox (500 mg orally) and/or $NaHCO_3$ (44 mEq/liter) was given prior to MTX administration to ensure proper urinary alkalinization. A urinary pH of > 6.5 was required before receiving therapy. Vincristine (1.4 mg/m^2, not to exceed a total dose of 2 mg) was given as an iv bolus during the middle of the MTX infusion. MTX was administered as a 4-hour infusion in 2 liters of 5% dextrose in water at doses ranging from 50 to 300 mg/kg of body weight. The CF rescue began 4 hours following the completion of the MTX infusion (8 hours after the start of infusion). An initial dose of 40 mg/m^2 was given as an iv bolus push, followed by 11 doses of 15 mg im or orally at 6-hour intervals.

Blood (plasma) samples were drawn at 0, 2, 4, 8, 12, 24, 48, and 72 hours after beginning the MTX infusion. Following the start of the MTX infusion, hourly fractional urines were obtained from 37 noncatheterized patients for the initial 8 hours, followed by a cumulative 8–24-hour collection. Urine volumes were carefully measured. All blood and urine specimens were analyzed for MTX. Creatinine concentrations were determined in the blood specimens only. MTX determinations were assayed by the competitive protein-binding method developed by Meyers et al (14) and were available for evaluation within 1½ hours of the blood sample being drawn.

If the plasma-MTX concentration exceeded 10^{-5} M at 24 hours, the patients were assumed to be at high risk with regard to developing severe myelosuppression (12,15). Consequently, these patients were placed on an intensified CF rescue schedule at this time, receiving 400 mg of CF by infusion over the next 24 hours, followed by 100 mg/24 hours until the plasma-MTX concentration reached 1×10^{-7} M.

Of the 239 infusions from which data were available, 172 were used to develop the plasma pharmacokinetic analysis presented in this report. Data from the 67 other courses were not included in the present analysis because the proper protocol was not followed exactly, doses and infusion times other than those listed above having been employed. The 172 courses used and analyzed were distributed by dose as shown in table 1. The number of infusions

TABLE 1.—No. of infusions administered at specific doses

Dose (mg/kg)	No. of infusions
50	61
100	37
150	45
200	29
Total	172

TABLE 2.—No. of infusions per patient

No. of infusions per patient	No. of patients	Total No. of infusions
1	33	33
2	26	52
3	13	39
4	6	24
5	2	10
6	1	6
7	—	—
8	1	8
Totals	82	172

received per patient is shown in table 2. Where patients received multiple infusions, doses were always escalated if changed from the previous infusion. The interval between multiple infusions varied from 1 week to 1 month.

CLINFO (16), a new prototype computer system dedicated to the systematic collection of clinical research data and its timely analysis, was used to generate a time-ordered data base of MTX-plasma values for each patient. The data were retrieved and organized into CLINFO worksheets for exploratory analysis using the system's analytic, plotting, and data manipulatory subroutines. Data sets were transcribed onto magnetic tape for the nonlinear regressions which were carried out on the Rand IBM 370/158. The regression programs employed in the data analysis were part of the nonlinear option provided in the Time Series Processor (TSP) of the Harvard Computation Center and the Harvard Institute of Economic Research (17).

RESULTS

Plasma Clearance

The plasma-MTX values used in assessing plasma clearance were those determined at 4, 8, 12, 24, 48, and 72 hours after the beginning of infusion. While most patients had their samples withdrawn for analysis at these times, there were some whose values were obtained at other time points, generally within 1–2 hours of those noted. The large number of concentration values and the relatively large number of time points at which concentration values were measured provide a statistically meaningful description of MTX-plasma decay in HDMTX/CF therapy.

The common logarithms of the concentrations were plotted as a function of the actual time of sample collection for each dose (eg, fig 1). The decay curve suggested a two-compartment pharmacokinetic model. A biexponential description of the time course of the plasma-MTX level using the function

$$y = b_1 e^{-a_1 t} + b_2 e^{-a_2 t} \quad [1]$$

was found to adequately describe the MTX-plasma concentration. A third compartment was not found to be necessary. Here y is the molar concentration of MTX and t is the time measured from the beginning of infusion. Because our data extend only to 72 hours, the equation is a valid description of decay only up to this time. The parameters were evaluated by recasting the equation in the form

$$\log y = \log (b_1 e^{-a_1 t} + b_2 e^{-a_2 t}) + E, \quad [2]$$

where a_1, a_2, b_1, and b_2 are constants to be determined and E is the error. By means of the nonlinear regression program in the TSP (17), the equation was solved for the values which minimized the objective function $\sum_i [\log y(t_i) - \log y_i(t_i)]^2$. Plots of individual therapeutic courses showed that the spread in the concentration values at any time point represented differences between patients and not assay measurement error, and that this spread was most evenly distributed in the space defined by log y versus t. By regressing on the logarithm of the concentration, we were able to assure convergence of the least squares analysis.

The input data are summarized in table 3. An average plasma-MTX concentration at each tabulated time point is given as the molar-plasma concentration. This value was calculated by solving the biexponential regression equation at each time point shown. Plasma-MTX values constituted the raw data at different time points but since not all the patients had samples taken at each of the listed times, the number of drug measurements actually made differs from time to time. We have therefore given the number of measurements available at each time point as the figure in parentheses for each concentration in table 3. The number of measured concentrations pertains in three instances to the measurements made over a range of time. The three instances are noted in the table and are the 20-hour (range, 20–22 hours), 48-hour (range, 44–48 hours), and 72-hour (range, 69–72

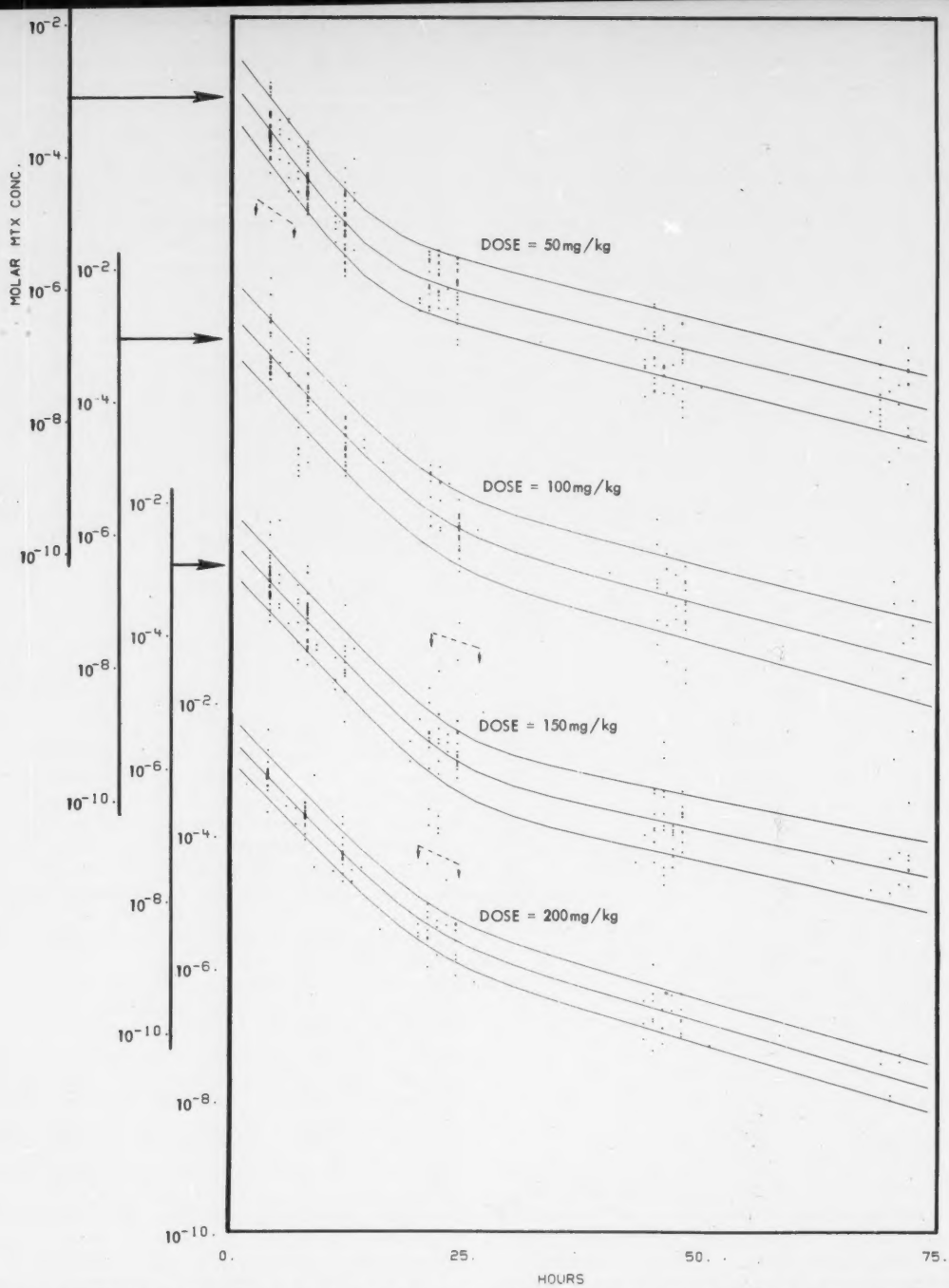

FIGURE 1.—MTX-plasma clearance curve for doses of 50, 100, 150, and 200 mg/kg. A biexponential fit to serum concentration values was obtained by least squares nonlinear regression analysis. Upper and lower curves represent the 1 SD error limits. The points show individual patient measurements.

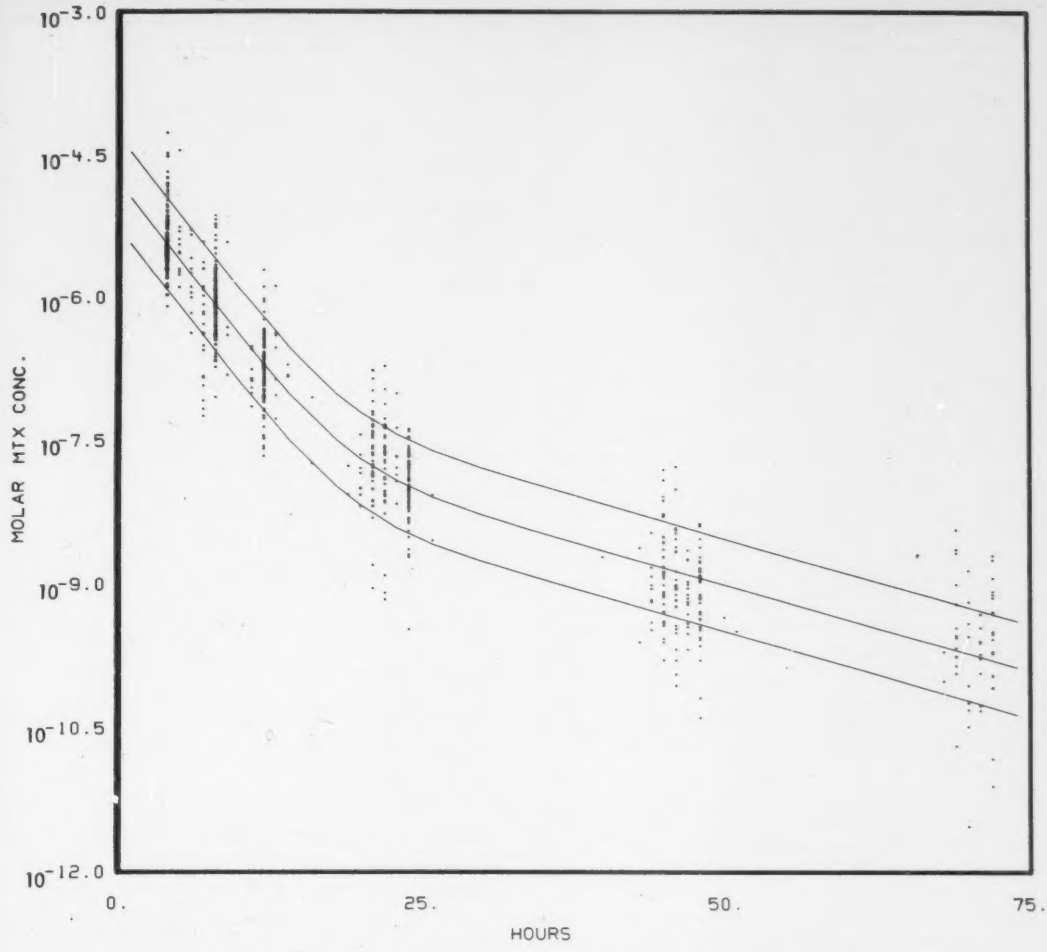

FIGURE 2.—MTX-plasma clearance curve for a unit dose of 1 mg/kg. This was derived by first scaling all data points by dose to 1 mg/kg and then performing a nonlinear regression. Upper and lower curves are 1 SD limits, and the points show individual patient measurements.

hours) time points. Because all concentration values enter into the regression analysis, the total number available over the range gives the best description of the data base upon which the regression is computed.

The results of the regressions for each dose are summarized in tables 4 and 5. Table 4 gives the best values for the parameters that appear in equation [1]. The half-lives for each phase of the decay curve have been computed from each exponential member of equation [1], eg, $T1_i = \ln 2/a_i$, and are listed in table 5. The parameter values in the last row of tables 4 and 5 have been determined by regression on the aggregated data from all doses, the aggregation having been accomplished by scaling each experimental concentration by dose to a unit dose of 1 mg/kg. The R^2 value is the coefficient of determination and represents that fraction of the sample variance about the mean accounted for by the regression. The errors tabulated for a_1, b_1, a_2, and b_2 have been estimated numerically during the TSP nonlinear fit, and rep-

TABLE 3.—Input data*

Dose (mg/kg)	MTX-plasma concentration (No. of measured values)						
	4	8	12	20†	24	48‡	72§
	Time (hrs)						
50	1.70×10^{-4} (34)	3.16×10^{-5} (39)	6.76×10^{-6} (26)	1.05×10^{-6} (34)	6.76×10^{-7} (23)	9.12×10^{-8} (43)	1.29×10^{-8} (27)
100	3.72×10^{-4} (31)	8.91×10^{-5} (22)	2.24×10^{-5} (22)	2.04×10^{-6} (14)	8.91×10^{-7} (19)	7.59×10^{-8} (28)	9.55×10^{-9} (9)
150	6.46×10^{-4} (35)	1.58×10^{-4} (28)	3.98×10^{-5} (11)	3.24×10^{-6} (21)	1.29×10^{-6} (13)	1.38×10^{-7} (32)	2.63×10^{-8} (9)
200	7.08×10^{-4} (22)	1.86×10^{-4} (19)	5.01×10^{-5} (14)	5.37×10^{-6} (15)	2.46×10^{-6} (9)	1.82×10^{-7} (23)	1.86×10^{-8} (8)

*Average MTX concentration is presented as a function of time after infusion for various doses. The average values have been determined from the 2-compartment biexponential functions given in the text. At each time point the raw data consisted of the No. of MTX concentration values given by the No. in parentheses. The MTX concentration is expressed as the molar concentration.

†Patient Nos. summed over the 20–22-hr range.

‡Patient Nos. summed over the 44–48-hr range.

§Patient Nos. summed over the 69–72-hr range.

TABLE 4.—Values of parameters for biexponential fit to plasma-MTX concentration curves for various doses ($y = b_1e^{-a_1t} + b_2e^{-a_2t}$)

Dose (mg/kg)	b_1 (M)	a_1 (hrs^{-1})	b_2 (M)	a_2 (hrs^{-1})	R^2*
50	$9.54 \pm 2.60 \times 10^{-4}$	0.437 ± 0.037	$4.57 \pm 1.26 \times 10^{-6}$	0.0817 ± 0.0039	0.87
100	$1.53 \pm 0.46 \times 10^{-3}$	0.358 ± 0.037	$4.93 \pm 3.48 \times 10^{-6}$	0.0869 ± 0.0150	0.88
150	$2.60 \pm 0.58 \times 10^{-3}$	0.352 ± 0.025	$3.78 \pm 2.67 \times 10^{-6}$	0.0690 ± 0.0130	0.92
200	$2.77 \pm 0.65 \times 10^{-3}$	0.344 ± 0.028	$1.67 \pm 0.86 \times 10^{-5}$	0.0944 ± 0.0097	0.95
All doses (unit dose, 1 mg/kg)	$1.63 \pm 0.24 \times 10^{-5}$	0.379 ± 0.019	$6.31 \pm 1.49 \times 10^{-8}$	0.0829 ± 0.0047	0.89

*R^2 is the proportion of the variance explained by the regression.

TABLE 5.—Values of the first and second half-lives of the 2-compartment model ($y = b_1e^{-a_1t} + b_2e^{-a_2t}$) for different doses

Dose (mg/kg)	1st half-life ± SD (hrs)	2nd half-life ± SD (hrs)
50	1.59 ± 0.13	8.48 ± 0.60
100	1.94 ± 0.20	7.98 ± 1.40
150	1.97 ± 0.14	10.05 ± 1.89
200	2.01 ± 0.16	7.34 ± 0.75
All doses (unit dose, 1 mg/kg)	1.83 ± 0.09	8.36 ± 0.47

resent 1 SD of the parameter mean. The errors for the first and second half-lives also represent 1 SD and have been calculated as the propagated error in half-life resulting from the error in the a parameter (18, *see* page 98).

The experimental MTX concentrations and best biphasic fit are shown for each dose in figure 1. The $\log_{10}$ of the molar-MTX concentration has been plotted against time and individual patient results appear as the points on the graph. Figure 2 shows the regression results when all data are aggregated by scaling each data set by dose to a unit dose of 1 mg/kg.

The lines on either side of the best biphasic fit in figures 1 and 2 give the standard error of the regression estimate *for an individual observation.* This error σ_{1y} has been calculated by first applying the propagation of errors equation (18, *see* page 98) to equation [2] to determine $\hat{\sigma}^2_{1y}$, the variance of the predicted mean value of $\log_{10} y$ at a point,

$$\hat{\sigma}^2_{1y} = \{[e^{-2a_1t}(\sigma^2_{b_1} + b_1^2t^2\sigma^2_{a_1}) + e^{-2a_2t}(\sigma^2_{b_2} + b_2^2t^2\sigma^2_{a_2})]/(b_1e^{-a_1t} + b_2e^{-a_2t})^2\}/\ln^2 10 \quad [3]$$

and then adding to this value the standard variance of the regression estimate, σ^2, to yield σ_{1y} according to

$$\sigma_{1y} = \sqrt{\bar{\sigma}^2_{1y} + \sigma^2} \quad [4]$$

(19). The σ^2 values are 0.25, 0.30, 0.20, 0.11, and 0.23 for the 50-, 100-, 150-, and 200-mg/kg and unit-dose cases respectively.

As can be seen from the half-life values in table 5, the regression curves for all doses have the same shape. In particular, the $T1_{\frac{1}{2}}$ and $T2_{\frac{1}{2}}$ values do not differ significantly by the t statistic from the values obtained from the aggregated unit-dose data set at the 95% confidence level. Although not shown here, error bands similar to those in figure 1 have been calculated for the same individual dose data sets but with all concentrations scaled by the appropriate dose to 1 mg/kg. At the 95% confidence level, each regression band overlaps the fit to the aggregated unit-dose data throughout the entire time range investigated, demonstrating that no significant difference exists among the predicted estimates of plasma decay as a function of dose. This confirms that all of the data presented here can be described by a single biphasic model (equation [1] with the unit-dose parameters of table 4), provided that it is appropriately scaled by dose.

Finally, for each dose we determine the fraction of patients undergoing therapy according to the present protocol who are expected to require intensive CF rescue. This may be calculated from the value of the variance at 24 hours and the intensified rescue threshold value of 10^{-5} M. The distribution of the logs of the serum-MTX concentration value at 24 hours is a Gaussian function characterized by a mean equal to the value of the regression line at this point (log y) and the 24-hour variance. The portion of this distribution characterized by concentration values $> 10^{-5}$ M gives the fraction of patients expected to require intensive rescue. This fraction is 0.009 at 50 mg/kg, 0.027 at 100 mg/kg, 0.024 at 150 mg/kg,[6] and 0.030 at 200 mg/kg. In earlier work (12) where a lower threshold value of 5×10^{-6} M at 24 hours was employed, it was found that nine of 111 patients (8%) actually underwent intensified rescue. This compares well with the predicted value of 10%, using the above computational procedure.

Kidney Clearance

The cumulative masses of MTX excreted in the urine over the time intervals, 4–8 and 8–24 hours after start of infusion, were calculated from the fractional urine data. Using corresponding plasma-MTX concentration values, kidney clearance rates were calculated for individual patients for the ranges 4–8, 8–24, and 4–24 hours. These ranges were selected to investigate whether kidney clearance changed as time progressed after the end of infusion. If MTX crystallization in the kidney was present, one might expect to see a noticeable decline in the clearance rate at long times.

The kidney clearance rate k was calculated for each patient from the relation

$$C_uV = \int_{t_1}^{t_2} kC\,dt \quad [5]$$

where C_uV is the cumulative urinary excretion of MTX over the time interval (t_2-t_1), C_u is the concentration of drug in the urine, V is the volume of urine collected over the time interval, and C is the concentration of MTX in the plasma. The integral representation is required since the plasma-MTX concentration changes by as much as two decades over the collection interval. The functional form assumed to hold for C was a single exponential $\alpha \exp(-\beta)$, since all data were collected during the first 24 hours after start of infusion where, as shown above, one exponential adequately describes the plasma clearance. The two plasma points at the ends of the collection interval were used to determine the values of the parameters α and β.

The results are shown in table 6. It can be seen that each time period provides an estimate of the kidney clearance rate but that the values so obtained are not statistically different from one another. The data as a whole, however, do provide a good measure of the average MTX-kidney clearance rate, 104 ± 8 ml/minute. This figure is quite close to the 125-ml/minute value usually accepted for inulin clearance in a 70-kg man and is somewhat lower than the early 190-ml/minute estimates of MTX-kidney clearance (20).

An attempt has been made to study data from patients with reduced rates of plasma clearance in order to determine if their kidney clearance is significantly lower than the average, particularly at long times after infusion. Unfortunately our present data base neither contains sufficient numbers of these slow-clearing patients nor possesses sufficiently accurate urine clearance data for statistical clarity.

It should also be noted that during the collection of these data, < 60% of the injected MTX was usually recovered in the urine by 24 hours. Whether the remaining MTX is lost in feces, is metabolized, or is slowly released from a deep compartment is

[6]The apparent reversal of expected probabilities for the 100- and 150-mg/kg cases is a statistical artifact resulting from the large variance obtained from the 100-mg/kg data.

TABLE 6.—Kidney clearance rates computed for various urine collection times

Collection interval (hrs)	No. of patients	Kidney clearance rate (ml/min)			
		Minimum	Maximum	Mean	SE of mean
4–8	18	33.0	404.6	122.6	23.8
8–24	33	20.6	207.0	95.3	8.9
4–24	22	30.0	260.1	100.9	13.1
		Overall mean = 103.7 ± 8.1 ml/min			

TABLE 7.—Urine excretion of MTX during high-dose therapy (% of administered dose excreted)

Time of collection (hrs)		Dose administered (mg/kg of body weight)*					
		50	100	150	200	250	300
0–4	Mean	21% (1)	18% (10)	20% (8)	21% (15)	19% (6)	14% (6)
	SD		8	8	6	7.5	3.5
0–8	Mean	34% (13)	33% (13)	40% (9)	40% (15)	36% (7)	35% (6)
	SD	20	15	18	10.5	7	8.5
8–24	Mean	12% (12)	11% (12)	16% (9)	17% (15)	11.5% (7)	12% (6)
	SD	7	6	11.5	14	3.5	6.4
0–24	Mean	44% (14)	46.5% (13)	57.5% (9)	57% (15)	48.5% (7)	48.5% (6)
	SD	20	15	19	18	9	13

*Nos. in parentheses = No. of patient courses studied.

not resolved by the present data, but the low level of recovery does agree with other recent results (21).

Table 7 further examines MTX renal excretion for the 24 hours following initiation of the MTX infusion. It may be seen that over the range of 50–300 mg/kg there is no difference in the percent of administered dose excreted in the time periods studied. These data tend to support our observations that plasma-decay rates are independent of dose administered (tables 3 and 4).

DISCUSSION

Data of the type reported here should add reliability to the prediction of MTX clearance and thus aid clinicians in attempts to avoid excessive toxicity in HDMTX/CF therapy. It has been reported earlier that infused patients who are likely to encounter severe myelosuppression (wbc count $< 2000/mm^3$, platelet count $< 100{,}000/mm^3$) have threshold plasma-MTX levels $> 5 \times 10^{-6}$ M at 24 hours (12) or 5×10^{-7} M at 48 hours (22) after start of infusion. A physician employing the same dose range and protocol used here (prior to intensified rescue) can refer to the expected range of plasma measurements as shown in figure 1, and can use this to flag possibly erroneous data points or to identify patients who are clearing slowly enough to have plasma levels in excess of these threshold values and place them on intensified hydration and rescue therapy.

It should be noted, however, that in previous studies, our intensified rescue protocol only achieved a moderately significant reduction in toxicity over that encountered by patients undergoing a single level rescue protocol of 15 mg CF im at 6-hour intervals (a significance level of $P = 0.12$ was obtained in a roughly dose-matched comparison [10,12]). This demonstration of toxicity reduction, albeit somewhat weak, did however lead to the decision to not exclude future patients from the potentially improved intensified rescue protocol. Hence no appropriately matched control group was available for comparison in the present study and no increased significance of toxicity reduction over earlier results could be demonstrated.

The present results on kidney clearance reveal no statistically meaningful changes in the clearance rate during the 20-hour time period following the end of infusion. If crystallization of MTX in the tubules is occurring in some patients, the clearance data are not precise enough to allow the effect to be identified. Because the present data were obtained from patients who voluntarily voided their bladders at fixed times after infusion, it cannot be assured that all urine present is fully excreted and that a residual urine volume does not increase the drug concentration of the next urine sample. Such urine retention could severely affect the results of

our fractional urines and thus serve to mask any time-dependent clearance rate that may actually be present. This observation, combined with the lack of statistical significance obtained in our serial clearance calculation, suggests that improved data may be obtained in the future from catheterized patients.

It has been reported (21,23) that plasma-decay curves may be triphasic. In the case of the present infusion data, only a biphasic character is discernible. That such a biphasic curve is sufficient to fit the data is indicated by the high value of R^2 for all dose studies. Furthermore, if (log y – log y_c) residuals are plotted for all data points against the time variable, positive and negative values are found to be distributed evenly over all times, thus indicating no systematic deviation from the biexponential fit. The triphasic results reported by Leme et al (21) were obtained from patients given rapid iv injections and not the 4-hour infusion employed here. It is therefore possible that the first part of the triphasic decay curve of Leme et al is attributable to the redistribution of MTX throughout all the tissue spaces immediately after injection, and that this distribution phase is not seen in our data since it occurs during the 4 hours of infusion preceding the plasma-clearance data.

It can be seen that the first half-life ranges between 1.6 and 2.0 hours while the second ranges between 7.3 and 10.0 hours. These half-lives properly describe the plasma-MTX decay curves only for the present protocol since this protocol involves significant hydration, urine alkalinization, and diuresis; identical procedures have not been employed in other studies. Nevertheless, in the interest of assessing our estimates of half-life, it is still instructive to compare them to similar results obtained under other related protocols. The closest comparison that can be made is to the results of Stoller et al (15), who employed 6-hour iv infusions of MTX together with fluid loading and urine alkalinization. They reported biphasic decay curves with dose average half-lives of 2.06 ± 0.16 and 10.4 ± 1.8 hours which compare favorably with our dose values of 1.83 ± 0.09 and 8.36 ± 0.47 hours. The comparison is particularly apt since those authors actually fit their data with a biexponential model, as we have also done.

Leme et al (21) and Huffman et al (23) reported second half-life values of 3.3 and 3.5 hours respectively, which roughly correspond with our first half-life value of 1.83 hours. Exact agreement has not been found, nor is it expected, since Leme et al and Huffman et al have both employed different protocols and have numerically analyzed their data using mathematic representations of pharmacokinetic behavior different from the biexponential form used by us. Huffman's third half-life, corresponding approximately to our 10.4-hour value, was considerably larger with a value of 27 hours. As pointed out by Stoller et al (15), Huffman's radioassay technique detects all MTX metabolites and may therefore not be measuring the same plasma clearance as determined here by the competitive binding assay.

A traditional view of the biphasic nature of the post-infusion MTX decay is to assume that there are two lumped body regions that contribute to the decay, a rapidly mobilizable one and a second slowly mobilizable one. Such compartments can only rarely be uniquely identified with organs and physiologic function, particularly in the case of a drug such as MTX that exhibits nonlinear transport (24,25). It is our goal to eventually construct a pharmacokinetic model of MTX which incorporates measures of physiologic function that may be measured in the laboratory and may vary from individual to individual. Such a model will be of the Bischoff, Dedrick, and Zaharko physiologic multi-compartment type (20,24,26). Calculations performed on a version of this model applied to MTX have strongly demonstrated the need for more information on MTX clearance paths other than kidney clearance (particularly biliary recirculation, MTX metabolism, and fecal clearances) in order to fully understand the shape of the plasma-decay curves. Because gut clearance parameters may play a dominant role in the second part of the decay curve, our present analysis has been confined only to the classic pharmacokinetic description of average plasma clearance.

It is expected that the plasma and urine clearance data obtained here will eventually be useful in the construction of an improved physiologic pharmacokinetic model. Our plasma-decay curves have been obtained from a large number of infusions: 172 at the time of the regression analysis. By contrast, the recent pharmacokinetic regression results of Leme et al (21), Reich et al (27), Stoller et al (15), and Tattersall et al (22) depended on eight, nine, seven, and 18 courses of infusion therapy respectively. The larger number of courses here has allowed us to investigate plasma clearance by dose and to show that no meaningful dose dependence exists over the 50–200-mg/kg range. It is likely (from our urine excretion data) that this observation is consistent with doses up to 300 mg/kg. This validates the unit-dose concept and thus assures us that the resulting plasma-decay curve is a statistically strongly based function upon which to build future pharmacokinetic models.

It should be recognized, however, that important differences in tissue exposure to presumed toxic

and therapeutic drug concentrations are noted as the MTX dose is increased. For example (fig 1), the duration of plasma concentrations at $\geq 10^{-5}$ M is 11 hours at a dose of 50 mg/kg while there are 17 hours of similar drug concentrations at 200 mg/kg. It will be important to relate this observation to models and experimental data on normal and tumor tissue drug concentration.

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Twice Weekly 5-Azacytidine Infusion in Disseminated Metastatic Cancer: A Phase II Study [1,2]

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SUMMARY

A phase II study of 5-azacytidine given twice weekly as a rapid iv infusion was performed on 116 patients with different metastatic cancers or refractory lymphomas at a dose of 150 mg/m² twice weekly × 6. Ninety-one patients were evaluable. Dose modifications were carried out depending on previous treatment status. Nausea and vomiting was a major side effect; significant granulocytopenia was observed in 35 patients. Responses were observed in only four patients. Our results indicate little effectiveness of this drug. The severe toxicity prevented escalation to potentially more effective dose levels.

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The pyrimidine analog, 5-azacytidine, has been shown to be an effective antineoplastic agent in several tumor systems in experimental animals (1–3). Responses in metastatic cancers have been few (4,5). However, the drug does have activity in acute leukemia (6–8). The studies in L1210 tumor systems indicated that the most effective schedule of administration was by continuous treatment given daily for 4 days, resulting in a 240% increase in lifespan. However, when given every fourth day there was a 207% increase in lifespan indicating considerable activity on that schedule. The Southeastern Cancer Study Group completed a phase I study of twice weekly 5-azacytidine (9); this is a report of a phase II trial in a spectrum of tumors.

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MATERIALS AND METHODS

Patients with disseminated cancer unresponsive to usual modalities of treatment were considered eligible for study provided that their life expectancy was judged to be > 6 weeks and that they had not received more than one prior chemotherapy program, except for patients with lymphoma who were entered in this study only if they were refractory to MOPP or to other forms of equivalent combination chemotherapy. Normal bone marrow and renal and hepatic functions were required. The pretreatment evaluation consisted of a complete clinical history and physical examination with careful measurements of tumor parameters. The following studies were done on all patients: complete blood cell counts; bone marrow; liver profile including transaminases, bilirubin, total proteins, and A/G ratio; serum alkaline phosphatase; and renal function studies including creatinine and BUN. All pertinent studies were repeated during treatment as follows: blood cell counts were performed prior to each dose (twice weekly) and liver function studies were performed once weekly.

Treatment Plan

Patients with metastatic cancers were given 5-azacytidine at a dose of 150 mg/m^2 twice weekly for 6 weeks which was administered by rapid iv injection dissolved in Ringer's lactate or normal saline. Since we had had very little prior experience with 5-azacytidine in lymphoma patients, we felt that reduced doses were indicated. All patients with lymphoma had had major prior therapy and were given 50 mg/m^2. No dose escalations were permitted.

Criteria of Response

Measurable tumors were followed carefully to assess any change. An excellent response (complete response) was defined as the disappearance of all evidence of disease. A good response (partial response) indicated > 50% reduction in measurable tumor. A fair response indicated a 25% reduction in tumor size. A questionable response was < 25% reduction.

Toxicity

The hematologic toxicity was judged as follows: *Hemoglobin level*—grade 1 = a fall of 3–4.9 g/100 ml and grade 2 = a fall of ≥ 5 g/100 ml. *Granulocyte count*—grade 1 = a fall to < 1500/mm^3; grade 2 = a fall to < 750/mm^3; and grade 3 = life-threatening granulocytopenia with infection. *Platelet count*—grade 1 = a fall to < 100,000/mm^3; grade 2 = a fall to < 50,000/mm^3; and grade 3 = life-threatening.

Other toxicity was graded as follows: *Hepatic function*—grade 1 = a 50% rise of SGOT or alkaline phosphatase above the normal levels and grade 2 = a 100% rise above normal levels. *Renal function*—any rise in BUN or creatinine above normal values was considered evidence of renal toxicity. *Gastrointestinal effects*—nausea and vomiting was graded as mild, moderate, or severe by each investigator.

RESULTS

There were 116 patients entered in this study and 91 were considered evaluable. Of the 25 unevaluable patients, three had an ineligible diagnosis, six had more than a single prior chemotherapy program, seven refused further treatment, five received incorrect doses of 5-azacytidine, one died of intercurrent disease, and three had inadequate data for evaluation. The responses are shown in table 1. Of the 91 evaluable patients, there were only four responses; two were judged as partial response (one large cell carcinoma of the lung and one renal cell carcinoma) and two were judged as fair response (one adenocarcinoma of the lung and one melanoma). Only seven patients with lymphoma were entered at reduced doses and none responded.

Table 2 shows the major toxic side effects. Severe nausea and vomiting occurred in 78% of the patients. Granulocytopenia was noted in 35 patients and was life-threatening in six. Two patients died of infection with low granulocyte counts (< 750 and < 1500/mm^3). Significant thrombocytopenia was observed in six patients and in two the count was < 50,000 cells/mm^3. Two patients had hemoglobin drops of ≥ 5 g/100 ml and 11 had drops from 3 to 4.9 g/100 ml. There were other minor side effects—one case of hepatic dysfunction and one case of severe alopecia.

Fourteen patients died while receiving treatment, two from toxicity and 12 from progressive disease.

DISCUSSION

This study indicates that 5-azacytidine given on the twice weekly schedule has little effectiveness in disseminated cancer. Severe nausea and vomiting occurred in the majority of patients. Granulocyte toxicity, although significant, was not intolerable in most patients; other hematologic toxicity was mild.

Previous studies concerning the instability of 5-azacytidine in aqueous solution necessitated giving the drug rapidly after reconstitution (10). However, recent studies by Israili et al (11), have indicated that the drug is stable in Ringer's lactate and can be given by continuous infusions. When given in

TABLE 1.—Response to 5-azacytidine

Primary site	No. of patients— Entered	Evaluable	No. of responses
Breast	8	6	0
Renal cell carcinoma	12	10	1 partial response
Other urogenital	3	3	0
Colon	9	7	0
Pancreas	3	2	0
Stomach	4	3	0
Rectum	1	1	0
Lung			
Unspecified	2	2	0
Squamous cell	9	6	0
Adenocarcinoma	13	11	1 FR*
Undifferentiated	5	5	0
Small cell	3	2	0
Large cell	4	2	1 partial response
Melanoma	10	10	1 FR*
Head and neck	14	14	0
Sarcomas, soft tissue	6	3	0
Lymphomas	7	4	0

*FR = 25% reduction in measurable tumor.

TABLE 2.—Major toxic effects (91 evaluable patients)

Toxic effect	Grade*	No. of patients
Nausea and vomiting		79
Granulocytopenia	1	9
	2	20
	3	6
Thrombocytopenia	1	4
	2	2
	3	0
Hemoglobin	1	11
	2	2
	3	0

**See* text for definitions.

such a manner, significant antitumor activity has been observed in acute leukemia (8).

Although sufficient numbers of patients were not entered in several of the various signal tumor categories to conclusively exclude activity, the gastrointestinal toxicity seemed to preclude any advantage of giving 5-azacytidine in this fashion. A recent publication would suggest that even continuous infusion of 5-azacytidine has very little activity in solid tumors (12). However, it does remain an active agent in acute leukemia.

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Suppression of Human Myelosarcoma Growth in Athymic Mice by a Primate Antiserum [1,2]

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SUMMARY

A primate (*Macaca speciosa*) antiserum prepared against the human chronic myelogenous leukemia cell line K-562 suppressed the growth of the human myelosarcomas in nude mice. The ip administration of 0.5 ml of immune serum plus 0.5 ml of guinea pig complement, starting 7 days after sc tumor transplantation, resulted in a fourfold to fivefold decrease in tumor weight at 15 days when compared to nude mice given pre-immune serum plus complement or complement alone. Whereas the other two groups experienced an exponential increase in tumor volume at 7–9 days after tumor transplantation, the immune serum-treated mice remained in a "lag" phase of tumor growth during which the tumor volume neither increased nor decreased substantially. Histopathologic studies revealed various degrees of tumor alterations ranging from focal hydropic cellular degeneration to massive coagulation necrosis. The incorporation of tritiated thymidine into the tumors was also markedly diminished in the mice given immune serum.

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Immunotherapy as a possible means of cancer treatment offers the theoretic advantages of a systemic treatment with a high degree of specificity and minimal toxicity that is lacking in conventional therapy regimens (1). Passive immunotherapy of tumors has yielded impressive results in experimental animals (2–7), but studies in man have been somewhat limited and less dramatic (8). One persistent difficulty with animal studies has always been the validity of analogies between human and animal tumors (1). Do results obtained with experimental animals really predict what might be expected when applied to human neoplasms? Most animal studies have focused on the response of animal tumors to various treatments. However, the use of athymic (nude) mice offers a compromise experimental model. Since these mice are congenitally athymic and lacking in cellular immunity (9), they do not reject transplanted human tumors (10). Thus, while this system uses an animal host, the tumor is of human origin.

We report the results of treatment of the human chronic myelogenous leukemia cell line K-562 (11) growing as sarcomas in nude mice with a primate (*Macaca speciosa*) antiserum prepared against these cells. The antiserum showed complement-dependent cytotoxicity in vitro (12) and suppressed the growth of myelosarcomas.

MATERIALS AND METHODS

A colony of nude mice with a BALB/c background has been maintained in our animal facilities for the past 4 years (13). Six-week-old mice, weighing approximately 15–20 g, were selected for use in the experiments.

The K-562 human myelogenous leukemia cells with the Philadelphia (Ph^1+) chromosome and a translocation 15:17 have been maintained in tissue culture for >6 years and have been previously described (11,14). The transplantation procedures for producing myelosarcomas from K-562 cells have been described in detail elsewhere (15,16). Briefly,

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tumor plugs (6 × 4 mm) were removed from myelosarcomas serially passaged in nude mice with a 6-mm biopsy trephine and were immediately transplanted sc into three groups of mice. Growth of the tumors was followed by measurement of the major and minor diameters of the myelosarcomas with a caliper three times weekly. The $Ph^{1}+$ myelosarcomas grew as well-vascularized spheroid to ellipsoid masses. Since the mean diameter can be calculated from the formula $d = \sqrt[3]{\frac{a^2b}{2}}$ where a is the major diameter and b is the minor diameter and since the volume of a sphere can be expressed by $V = \frac{\pi}{6} d^3$, the approximation formula $V = \frac{\pi}{12} (a^2b)$ was used to calculate the tumor volume.

A primate (*Macaca speciosa*) antiserum cytotoxic for the K-562 cell line, with a titer of 1:250, was given to mice in the experimental group. The titer was defined as the dilution which killed 50% of K-562 cells by the dye exclusion test. The preparation of this antiserum has been previously reported (12). Seven days after the transplantation of the tumors, ten mice received 0.5 ml of the undiluted antiserum and 0.5 ml of guinea pig complement (Colorado Serum Co, Denver, Col) ip. The control groups received either 0.5 ml of pre-immune serum plus 0.5 ml of complement or 1.0 ml of complement alone.

The injections were repeated on Days 8, 9, 12, and 13. On Day 14, the mice were given 2 microcuries/g of body weight of methyl-^{3}H-thymidine (^{3}H-TdR) with a specific activity of 25 millicuries/mmol ip. A second injection was given 12 hours later. The mice were killed 12 hours after the second injection and the tumors were removed and weighed. One half of each tumor was used for histology and the other half was digested in 5 ml of 10-X hydroxide of hyamine at 70°C for 4 hours. A 0.5-ml aliquot from each tumor was then used for liquid scintillation counting. Pieces of the tumors were randomly collected, fixed in 10% buffered formaldehyde, and embedded in paraffin. Sections were stained with hematoxylin and eosin, and with silver techniques for reticuline fibers.

RESULTS

The tumor volumes for the three groups of mice are plotted in figure 1. It has been a consistent observation in all of our studies that the myelosarcomas go through a lag period of approximately 7–9 days after transplantation before they begin to grow exponentially. During this latent period when vascularization is developing, the tumor volume actually decreases slightly. As can be seen from the figure, both the control group given complement alone and the group given pre-immune serum plus complement did begin to grow exponentially after 7–9 days, but the mice given immune sera remained in the lag period throughout the course of the experiment.

The histologic examination demonstrated that large portions of the tumors were replaced by areas of coagulation necrosis in which the K-562 cells appeared as acidophilic homogeneous masses, with loss of the nuclei (fig 2). The cytoplasmic outlines were preserved, leaving an empty space around the shrunken necrotic cells. In some mice, the whole tumor appeared as a solid, opaque nodule containing abundant debris (fig 3). A few specimens showed minimal changes characterized by groups of cells with various degrees of cytoplasmic hydropic degeneration, karyolysis, and/or karyorrhexis randomly distributed throughout the myelosarcomas (fig 4).

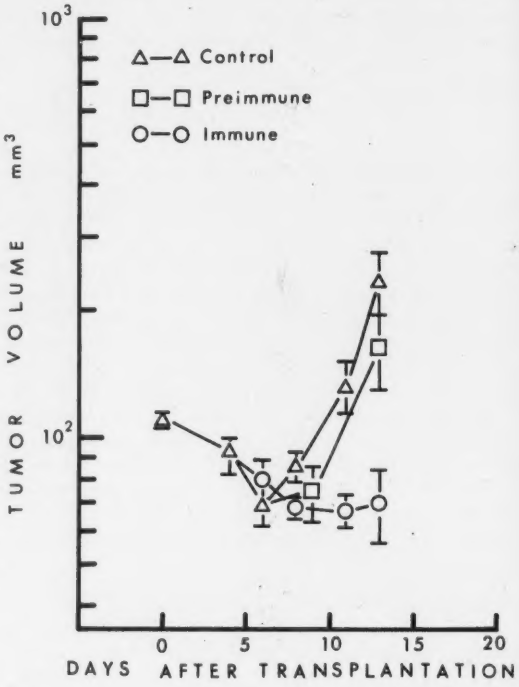

FIGURE 1.—Growth rate of serially transplanted myelosarcomas in nude mice. Each point represents the mean ±SE of 10 mice. Animals were injected on the 7th day after transplantation; control mice received normal guinea pig serum, whereas the other groups were given the normal or immune monkey sera plus the guinea pig serum.

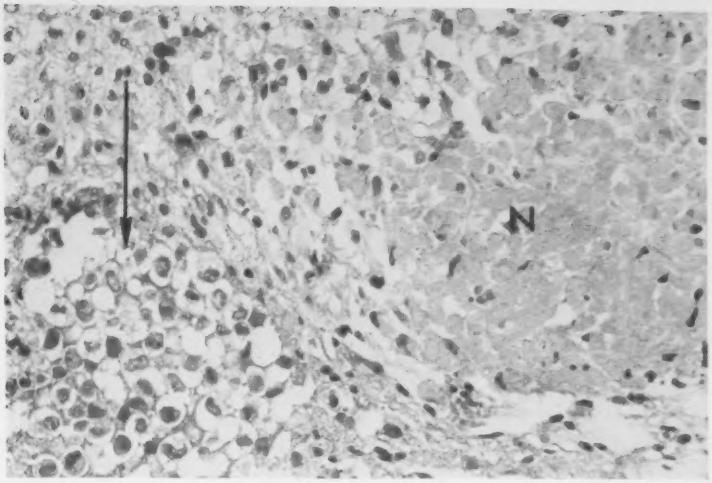

FIGURE 2.—Large area of coagulation necrosis (N) in a myelosarcoma after treatment with immune serum. Necrotic cells are barely seen as hyalin spheres without nuclei; adjacent area (arrow) shows hydropic degeneration of the tumor (hematoxylin and eosin, × 400).

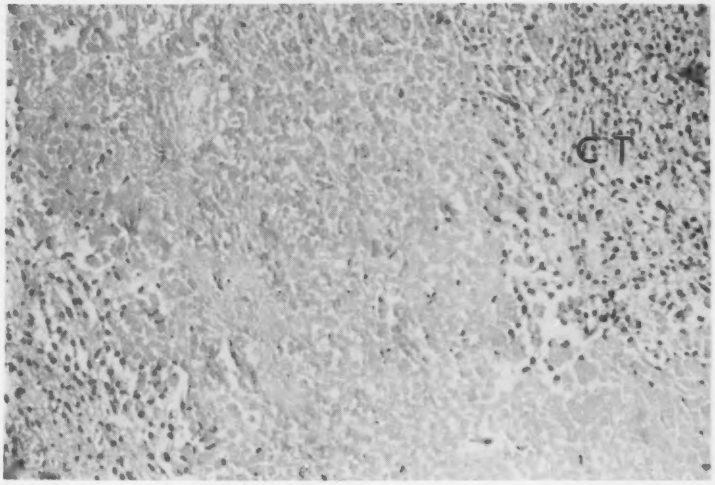

FIGURE 3.—Massive coagulation necrosis in a treated myelosarcoma. Connective tissue (CT) of the mouse surrounds the necrotic tumor (hematoxylin and eosin, × 120).

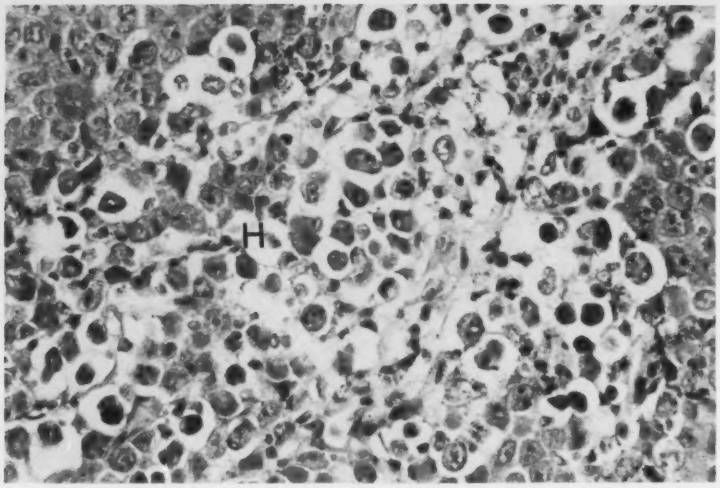

FIGURE 4.—Area of hydropic degeneration (H) in a treated myelosarcoma. Adjacent preserved tumor cells can be noted (hematoxylin and eosin, × 420).

Inflammatory signs were slight or absent, and vascular changes in the host tissues were minimal. Tumors from both groups of control mice did not show cellular modification (fig 5).

Data on the tumor weights and the incorporation of ^{3}H-TdR are presented in table 1. As can be seen, the tumors in the immune sera-treated group were roughly fourfold to fivefold smaller by weight and twofold to threefold smaller by volume when compared to the other two groups. Thus, the total radioactivity incorporated into the tumors was much lower in this group. The specific incorporation of ^{3}H-TdR was slightly less in the immune sera-treated mice than that in mice receiving pre-immune serum plus complement or complement alone.

DISCUSSION

The nude mouse has been increasingly used in various aspects of cancer research. It appeared that this mutant would be especially appropriate for immunotherapy experiments since human tumors can be maintained in an animal host (10,17,18) and most immunotherapy trials have used animal tumors which may not be strictly analogous to human neoplasms. Also, nude mice may more closely approximate the human situation since they lack cellular immunity and many patients have various degrees of immune system impairment.

The availability of a well-characterized human myelogenous leukemic cell line (11), the successful transplantation of these cells into nude mice (15), and the recent preparation of a primate antiserum against these cells (12) prompted studies on the passive immunotherapy of myelosarcomas. Since the antiserum demonstrated in vitro complement-dependent cytotoxicity, it was decided to administer exogenous guinea pig complement along with the antiserum to avoid complications arising from an insufficient amount of endogenous mouse complement.

The treatment of the myelosarcomas with the primate anti-K-562 antiserum suppressed tumor growth as evaluated by the parameters of tumor weight, volume, and incorporation of ^{3}H-TdR, and induced extensive cell necrosis as demonstrated by histopathologic examination. The arrest of tumor growth as determined by the gross volume was actually due to a partial or massive necrosis. However, the volume of the necrotic masses precluded a rapid removal by the macrophages of the host. Some mice showed only minimal histologic tumor changes, in spite of the high titer of the antiserum (1:250) and the relatively large doses. Several factors may be cited for the lack of regression. First, duration of treatment was relatively short because of the large quantity of antiserum required. Second, the K-562 myelosarcoma has proved to be extremely aggressive in mice. Thus, the ultimate fate of untreated nude mice bearing a myelosarcoma is death in cachexia when the tumor reaches a weight of 5–6 g on or after the sixth week after transplantation (15,19). In marked contrast, mice treated with the immune serum lived for at least 14–16 weeks while

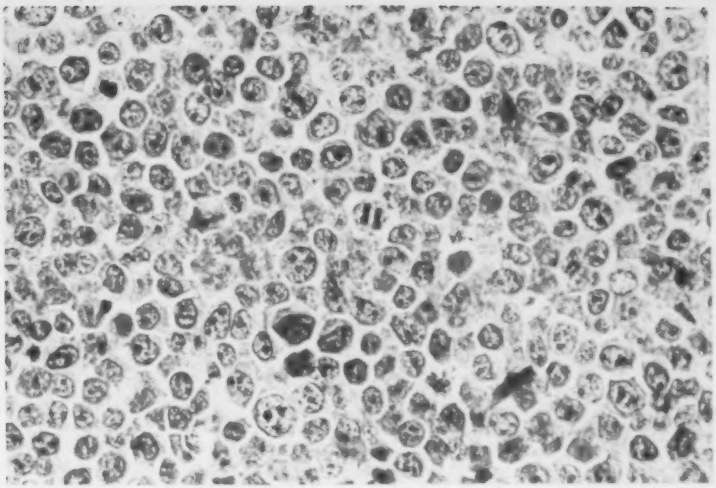

FIGURE 5.—Myelosarcoma growing in a control mouse. Characteristic homogeneous blast type of the cells and mitotic activity are preserved (hematoxylin and eosin, × 420).

TABLE 1.—^{3}H-TdR uptake by myelosarcomas made up of K-562 myelogenous leukemia cells*

Group	Tumor volume (mm^3)	Tumor weight (mg)	Specific ^{3}H-TdR incorporation (10^{-5} microcuries/mg)	Total ^{3}H-TdR incorporation (10^{-5} microcuries)
Control	236 ± 39	305 ± 61	10 ± 0.7	3158 ± 704
Pre-immune sera†	164 ± 34	235 ± 47	9 ± 0.4	2109 ± 448
Immune sera †	71 ± 14	64 ± 24	5 ± 1.4	543 ± 307

*Nos. = means ± 1 SE of 6–8 tumors rounded to the nearest unit.
†Plus guinea pig serum.

the necrotic tumor mass was being absorbed. Third, the initial tumor load at 7 days when treatment was started is much larger than that of most experimental immunotherapy models. We have found that nude mice immunologically reconstituted by a thymus take as long as 8 weeks to completely reabsorb the transplanted myelosarcomas (19). All of these factors would suggest that a very high antibody titer and/or a protracted period of treatment may be required. When mice bearing the myelosarcomas were treated with diluted primate antisera (titer 1:100) or an undiluted rabbit anti-K-562 antiserum (20) with a titer of 1:120, no effect on tumor growth was demonstrated. Again, this indicates a possible requirement for a high concentration of antibody and perhaps a high degree of specificity.

The fact that the primate antiserum was able to prevent the myelosarcomas from growing exponentially suggests that it might be possible to combine immunotherapy with other methods of treatment, such as chemotherapy, to achieve tumor regression via synergistic effects. This might also reduce the amount of antibody required. One approach which we are currently pursuing is the preparation of drug-antibody conjugates for possible tumor treatment.

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Treatment of Metastatic Breast Cancer With Adriamycin-Cyclophosphamide Induction Followed by Alternating Combination Therapy[1,2]

Martin D. Abeloff* and David S. Ettinger[3]

SUMMARY

Thirty-four patients with metastatic breast cancer and no prior chemotherapy were treated with an induction regimen of four courses of adriamycin-cyclophosphamide followed by a fixed sequence of three courses of methotrexate-5-fluorouracil alternating with each course of adriamycin-cyclophosphamide. In this protocol, adriamycin can be administered for a minimum of 18 months before the dose of 550 mg/m² is reached. The objective response rate was 56% (19 of 34 patients) with three complete responses and 16 partial responses. The median duration of response is 219 days and six of 19 patients remain in remission. The median survival of responders is 469 days while the nonresponders and progressors have a median survival of 273 days. The chemotherapy was administered in the outpatient department and was well tolerated. The scheduling of drugs in this study does not appear to result in significantly different response rates, duration of responses, or survival compared to those protocols in which these drugs are administered simultaneously.

[Cancer Treat Rep 61:1685–1689, 1977]

Combination chemotherapy has been shown to be highly effective in the management of advanced breast cancer (1). Despite a large number of clinical trials, the optimal multi-drug regimen has not been established. In 1974, Muggia et al reported encouraging results with a phase I and II evaluation of combination therapy with adriamycin and cyclophosphamide (2) in a variety of solid tumors. Jones et al subsequently reported an 80% objective response rate to the combination of adriamycin and cyclophosphamide in patients with advanced breast cancer (3). Interest in this very active regimen was further stimulated by studies from the Southern Research Institute (Birmingham, Ala) which demonstrated that the combination of adriamycin plus cyclophosphamide was therapeutically potentiating against four different C3H mammary tumor lines, the B16 melanoma, the Ridgway osteogenic sarcoma, and the P388 leukemia (4).

Although chemotherapy with adriamycin and cyclophosphamide appeared to be a rapid and safe means of inducing response in patients with advanced breast cancer, this regimen resulted in a complete response rate of only 12%, and the median duration of response was 10 months. The period of administration of this combination was limited to 10–12 months by the significant risk of adriamycin cardiotoxicity in patients whose cumulative adriamycin dose exceeds 550 mg/m². Thus, responding patients whose cumulative adriamycin dose reached 500–550 mg/m² started receiving another chemotherapeutic regimen (CMF) of cyclophosphamide, methotrexate, and 5-fluorouracil (5-FU) (5).

In an attempt to increase the complete response rate and prolong the duration of response, we designed a treatment for metastatic breast cancer which consists of an induction regimen of four courses of adriamycin and cyclophosphamide followed by a fixed sequence of three courses of

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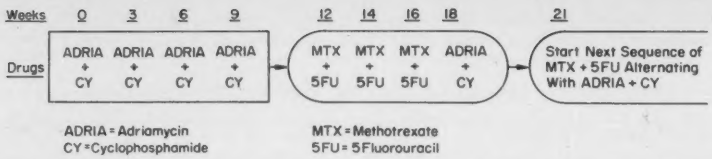

FIGURE 1.—Schedule of chemotherapy. Therapy is continued until progression or relapse occurs or until a cumulative dose of 550 mg/m² of adriamycin has been administered. When maximal dose of adriamycin has been reached, patient is given CMF regimen.

methotrexate and 5-FU alternating with each course of adriamycin-cyclophosphamide (fig 1). This protocol provides for the administration of adriamycin for a minimum of 18 months before the dose of 550 mg/m² is reached and the drug is discontinued.

MATERIALS AND METHODS

Between November 1974 and October 1976, 37 patients with metastatic breast cancer were admitted to the study. Eligibility criteria included no previous therapy with cytotoxic drugs and the presence of objectively measurable metastatic disease. Patients with inoperable local disease were eligible for this study. Osseous metastases alone were not considered adequate criteria for objective measurement of metastatic disease. Patients > 70 years old or patients with unstable cardiac disease were not eligible for this study. All eligible patients were admitted to this study.

Drug Regimen

Adriamycin (50 mg/m²) and cyclophosphamide (700 mg/m²) were administered iv every 3 weeks for four courses. This induction phase was followed by three courses of methotrexate (25 mg/m²) and 5-FU (600 mg/m²) iv every 2 weeks alternating with each subsequent course of adriamycin-cyclophosphamide (fig 1). Responding patients whose cumulative adriamycin dose reached 550 mg/m² were then given combination chemotherapy with CMF (5). Dose modification was primarily determined by the degree of bone marrow suppression at the time of drug administration. When the peripheral leukocyte count was > 5000/mm³ and the platelet count was > 100,000/mm³, 100% of the calculated dose was administered. Fifty percent of the calculated dose was administered when the leukocyte count was between 3000 and 5000/mm³ and/or the platelet count was between 75,000 and 100,000/mm³. If the wbc count was < 3000/mm³ and/or the platelet count was < 75,000/mm³, no drugs were administered and blood cell counts were repeated at weekly intervals until the leukocyte count was > 3000/mm³ and the platelet count was > 75,000/mm³. At that time, chemotherapy was restarted with doses determined by previously stated criteria.

During the first two courses of treatment, patients were seen and blood cell counts were determined at weekly intervals. If complications of therapy did not occur, the patients were subsequently assessed only on the days of administration of chemotherapy. Tumor measurements and appropriate chemistries were obtained at 3–4-week intervals and pertinent radiologic studies were repeated at 1–3-month intervals.

Criteria of Response

The minimal criterion for partial response was a decrease of 50% in the product of the two largest perpendicular diameters of measurable lesions for 1 month with stability or recalcification of osteolytic lesions. In the case of liver metastases, a 50% decrease in vertical liver span was the minimal criterion for a partial response. Complete response was defined as disappearance of all measurable lesions and recalcification of osteolytic lesions. Patients were classified as having no response or stability when a decrease of < 50% or an increase of < 25% over original measurements of the product of the two largest perpendicular diameters of measurable lesions was recorded with no change in osseous lesions. An increase of ≥ 25% over original measurements of the product of the two largest perpendicular diameters of marker lesions and/or the occurrence of new lesions and/or progression of osteolytic lesions indicates progression of the tumor.

Patient Population

Thirty-seven patients were treated with this drug regimen. Two patients were considered nonevaluable. One of these had a concomitant therapeutic oophorectomy and therefore her subsequent objective response could not be definitely attributed to the chemotherapy. The second patient received only one course of adriamycin-cyclophosphamide prior to receiving radiotherapy to the only measurable lesions. A third patient had bone marrow involvement as the only evidence of metastatic disease and was not eligible for this protocol.

TABLE 1.—Distribution of metastatic lesions and response by site in 34 patients

Metastatic lesion	Total No. of sites	No. of responses (%)
Soft tissue-skin	11	8(73)
Nodes	15	10(66)
Breast	6	4(66)
Liver	4	2(50)
Lung and pleura	17	7(41)
Osseous	19	0(0)

The median age of the 34 evaluable patients was 53 years (range, 36–69 years). Twenty-nine of the patients were postmenopausal. Of the five premenopausal patients, four patients had metastatic disease at the time of diagnosis and one patient had inoperable breast cancer. The median disease-free interval from the time of initial diagnosis to the first recurrence of breast cancer was 13 months. Eight of the 34 patients (24%) were inoperable because of locally advanced disease or metastatic disease at the time of diagnosis. Twenty patients (59%) had had prior endocrine therapy with only two objective responses. Twelve patients (35%) had had prior radiotherapy and no patients had had prior cytotoxic chemotherapy. Examination of the Karnofsky performance status (PS) at the initiation of chemotherapy revealed that ten patients carried on normal activity (PS 9), 15 patients were able to care for their own needs (PS 7–8), and nine patients required considerable assistance (PS $\leq$ 5), including seven patients who were severely disabled and required hospitalization.

The distribution of metastatic lesions and response by site is outlined in table 1. There were 72 evaluable lesions in 34 patients. Metastatic bone survey and bone scan were done in all patients, and a liver scan was performed in the presence of abnormal liver function tests or hepatomegaly. Bone marrow biopsies and aspirates were performed in only eight patients and were positive for tumor cells in five patients. The most common sites of involvement were bone, lung-pleura, lymph nodes, and soft tissue-skin.

RESULTS

Patients who received at least two courses of adriamycin-cyclophosphamide were considered evaluable in this trial. Nineteen of the 34 evaluable patients (56%) had a complete (9%) or partial (47%) response. In four patients (12%), stability of disease was achieved and in 11 patients (32%) progression occurred while therapy was being given. Following two courses of adriamycin-cyclophosphamide, 17 patients had already achieved an objective response while 13 patients had no change in tumor status and four had progressed. Maximal response to therapy was seen after four courses of adriamycin and cyclophosphamide in 18 patients, and only one patient had a further objective response to methotrexate and 5-FU. Two patients who progressed during the methotrexate and 5-FU phase of the protocol had symptomatic improvement and stabilization of disease when restarted on adriamycin and cyclophosphamide. One patient who progressed while receiving methotrexate and 5-FU had further objective response to adriamycin-cyclophosphamide.

The metastatic sites most responsive to this therapeutic regimen were soft tissue-skin, lymph nodes, breast, and liver (table 1). Four patients received this chemotherapy regimen as the initial treatment for inoperable primary breast cancer and two responded. Recalcification of osteolytic lesions was not seen in any patient in this series. Bone marrow involvement did not preclude responses in that four of the five patients with positive marrow biopsies had objective responses in measurable sites.

The median duration of remission is 219 days (range, 105–529+ days) with six of 19 patients currently in remission. The six patients who remain in remission have been receiving the chemotherapy for 189, 345, 469, 490, 493, and 529 days, respectively. The survival of the responders was significantly greater than that of the nonresponders and progressors at $P < 0.05$; the mean survival of responders is 411 days (median, 469 days; range, 189+–595 days) with 13 of 19 still alive while the mean survival of nonresponders and progressors is 316 days (median, 273 days; range, 50–806 days) with only five of 15 patients alive.

Therapy was administered in the outpatient department and the major toxic effects were leukopenia, nausea, vomiting, and alopecia. The median nadir of the wbc counts was 2300/mm^3, and there were only three episodes of wbc counts < 1000/mm^3. There were two episodes of fever associated with leukopenia and in one patient an axillary abscess occurred with a wbc count of 1600/mm^3. Thrombocytopenia was not a limiting factor with only one episode of a platelet count < 100,000/mm^3. The episodes of severe granulocytopenia and thrombocytopenia, fever, and infection all occurred in patients with bone marrow involvement. The five patients with marrow involvement received full doses of drugs during the initial cycle of therapy since the leukocyte and platelet counts were in the normal range. However, all of the patients with marrow involvement subsequently required dose reductions.

TABLE 2.—Comparison of combination chemotherapy regimens for advanced breast cancer

Drug combination*	Objective response (%)	Complete response (%)	Median duration of responses (mos)	Median survival of responders (mos)	Ref No.
CMF	56	11	8	18	8
AV followed by CMF	52	15	9	22	8
Intermittent CMFVP	40	8	10	14	9
Weekly CMFVP	59	19	8	14	9
FAC	73	14	8	15	10
AC-MF	56	9	7	16	This study

*C = cyclophosphamide, M = methotrexate, F = 5-FU, A = adriamycin, V = vincristine, P = prednisone.

There were no definite cases of cardiotoxicity. One patient developed ventricular bigeminy after 200 mg/m^2 of adriamycin. Adriamycin was discontinued and the patient was given the CMF combination. After relapse with CMF, the patient was treated again with adriamycin with no cardiac sequelae occurring. No drug-associated deaths occurred with this protocol.

DISCUSSION

It has been well established that combination chemotherapy is considerably more effective than single-drug therapy in controlling advanced breast cancer (1,6). A number of multi-drug therapies have been reported to induce remission rates of > 50%. However, the limitations of these regimens are similar in that the complete response rates are generally < 20% and the median duration of responses is approximately 10 months (7,8).

Adriamycin-cyclophosphamide has been reported to be a particularly effective induction regimen in advanced breast cancer. Jones and his colleagues suggested that these drugs may be synergistic against human breast cancer (3). The design of our study attempts to capitalize on the induction efficacy of adriamycin-cyclophosphamide and to subsequently increase the number of complete responses and duration of responses by the utilization of methotrexate and 5-FU in fixed alternating sequence with adriamycin and cyclophosphamide. Our protocol also allows for the use of the adriamycin combination for a longer period of time before adriamycin must be discontinued because of maximal dose limitations.

The results of our study are comparable to published results with a variety of combination chemotherapy regimens (table 2). Our response rate (56%) was less than the 80% reported by Jones et al with the adriamycin-cyclophosphamide combination. The dose schedule in our protocol is significantly different from that of the Jones et al study in which adriamycin is administered iv on Day 1 at a dose of 40 mg/m^2 and cyclophosphamide is given orally at 200 mg/m^2/day in divided doses on Days 3–6 of the 21–28-day cycles. However, four other trials of adriamycin and cyclophosphamide have now reported objective response rates of 32%, 44%, 54%, and 60% in advanced breast cancer (11–14). It is important to emphasize that comparisons between these chemotherapy studies are made difficult by differences in drug dosages and schedules, heterogeneous patient populations, and differences in criteria of response.

It was anticipated that methotrexate and 5-FU would add to the overall response rate and that the cycling of combinations of drugs would prolong the duration of response. With the exception of one patient, the methotrexate and 5-FU combination did not result in any objective regression of tumor in patients who had achieved a partial response or failed to respond to adriamycin-cyclophosphamide. This lack of efficacy might be explained by the scheduling and/or relatively low doses of methotrexate and 5-FU or the presence of a drug-resistant population of tumor cells following a partial response to adriamycin and cyclophosphamide.

Thus, despite the sequencing of these effective agents in this multiple-drug regimen, our response rate, duration of remission, and survival of responders do not appear significantly different from those in protocols in which these drugs are administered simultaneously. The relatively small number of patients in this study and the absence of a control group preclude any judgment regarding the value of prolonging the time of administration of adriamycin in a cohort of responsive patients.

There continues to be considerable interest in the sequential use of non-cross-resistant combinations to increase the response rate and duration of re-

sponse in metastatic breast cancer. Although the preliminary data were encouraging, Brambilla and colleagues failed to show any significant improvement in overall response rate, duration of response, and survival in advanced breast cancer with the sequential use of two non-cross-resistant combinations (adriamycin-vincristine followed by CMF) (8). Boston et al also did not show any difference in response rate or duration of response in a comparison of adriamycin-cyclophosphamide versus adriamycin-cyclophosphamide in sequence with methotrexate and 5-FU (14).

Our results support the observations of other investigators (7,8) that combination chemotherapy seems to have reached a plateau in its capacity to control metastatic breast cancer and that alternating regimens do not appear to increase the length of remission. However, further studies with appropriate control groups are needed to fully evaluate the efficacy of sequential use of non-cross-resistant combinations. The availability of new agents and the utilization of immunotherapy (10) may also aid in the development of more effective treatment programs in advanced breast cancer.

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Evaluation of a Hexamethylmelamine and 5-Fluorouracil Combination in the Treatment of Advanced Ovarian Carcinoma [1,2,3]

Carl G. Kardinal [4,*] and James K. Luce [5]

SUMMARY

The combination of two nonalkylating agents, hexamethylmelamine (HMM) and 5-fluorouracil (5-FU), was evaluated in 14 patients with advanced ovarian carcinoma. Nine patients had been treated previously with alkylating agents. The overall response rate was 36% which is not greater than could be expected with either HMM or 5-FU alone. However, the median survival of the five responders was > 24 months. Treatment had to be discontinued in five of 14 patients due to excessive toxicity. Although the toxic effects were predominately gastrointestinal, unpredictable hematopoietic and neurotoxic effects were observed.

[Cancer Treat Rep 61:1691–1693, 1977]

Alkylating agents such as melphalan and chlorambucil are currently considered the treatment of choice for advanced ovarian carcinoma. Response rates of 40%–50% have been well documented (1–3). Several drugs without alkylator activity are active in ovarian cancer, but combination nonalkylator therapy has not been evaluated. If such a combination has enhanced activity and tolerable toxicity, it could be very useful in patients refractory to alkylating agents or, perhaps, even in combination with alkylating agents.

Hexamethylmelamine (HMM) and 5-fluorouracil (5-FU) are both active as single agents in the treatment of advanced ovarian cancer. Although HMM has a chemical structure similar to the alkylating agent triethylenemelamine, it appears that HMM itself does not possess alkylator activity. HMM may act as a pyrimidine antimetabolite (4,5). The response rate of HMM in advanced ovarian cancer is approximately 40%, and the drug is active in patients who are refractory to alkylating agents (6,7).

5-FU is a fluorinated pyrimidine which blocks intracellular thymidylate synthetase. This drug has been evaluated extensively as a single agent in the treatment of advanced ovarian cancer (8,9). The response rate is in the range of 30%.

The primary purpose of this study was to determine the response rate and toxicity of a combination of two nonalkylating agents in the treatment of advanced ovarian cancer. It was anticipated that the two drugs could be used together in nearly full doses since the primary toxic effects of 5-FU are hematopoietic and the primary toxic effects of HMM are gastrointestinal and neurologic. A pilot study evaluating a combination of HMM and 5-FU that was conducted for the WCSG is reported here.

MATERIALS AND METHODS

All women with documented FIGO (International Federation of Gynecologists and Obstetricians) stage III or IV ovarian cancer were eligible for study. If patients had had surgery, 14 days must have elapsed and if they had had chemotherapy, > 21 days must have elapsed. Only patients with an index lesion evaluable for response were eligible for the study. Informed consent was obtained from each patient.

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[3]This pilot study was conducted for the Western Cancer Study Group (WCSG).

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The treatment program was as follows: 8 mg/kg of HMM orally on Days 1–21, 12 mg/kg of 5-FU iv on Days 1, 8, 15, and 22, no treatment on Days 23–42, and then the cycle was repeated. All patients who completed one cycle of therapy were considered evaluable for response and toxicity.

RESULTS

A group of 15 patients with advanced FIGO stage III or IV ovarian carcinoma were treated with the combination of HMM and 5-FU. One patient, a 59-year-old woman with mucinous cystadenocarcinoma, was considered unevaluable for response or toxicity since she received only 3 days of treatment and died 8 days later of advanced disease. All other patients completed at least one cycle of therapy and were considered evaluable. Of the 14 evaluable patients, nine had received prior chemotherapy with alkylating agents, seven had had prior radiation therapy, and five had received both prior alkylating agent therapy as well as irradiation. Three patients had received no prior treatment other than surgery.

Responses are defined as follows: a complete response (CR) is the disappearance of all clinical evidence of disease; a partial response (PR) is a reduction of > 50% of all measurable parameters of disease. Five of the 14 evaluable patients achieved either a CR or PR for an overall response rate of 36%. The response rate was essentially the same in patients who had received prior therapy with alkylating agents as in those patients who had not (table 1). Response by histologic type is listed in table 2.

The toxicity of this regimen was excessive. Treatment had to be discontinued in five of the 14 patients after one or two cycles of therapy because of severe gastrointestinal (GI) or hematopoietic toxicity. In four of 14 patients nausea and vomiting with or without diarrhea resulted in weight loss of > 7%–10%. Hematopoietic toxicity was unpredictable and was not related to prior radiation therapy. Leukopenia with a total wbc count of < 2000/mm^3 occurred in two patients. Thrombocytopenia of < 50,000 platelets/mm^3 occurred in three patients. Marrow suppression persisted for > 60 days after one cycle of treatment in one of these patients. This patient had not had prior irradiation. In all five of the responders a 50% dose reduction after one or two cycles of therapy was necessary due to hematopoietic and/or GI toxicity. In four of the five responders, tolerance to the GI toxicity seemed to develop, but one of the responders continued to have frequent and excessive vomiting. Transient peripheral neuropathies developed in two patients. In one patient neuropathy cleared after a 50% dose reduction; in the other patient it cleared after medication was discontinued. Depression requiring treatment with tricyclic antidepressants was observed in two patients.

The time to maximal response was delayed. Although some sign that the patients were going to respond could generally be detected after one or

TABLE 1.—Response by prior alkylating agent therapy

	No. of patients	No. of patients with—		Response rate (%)
		CR	PR	
All patients	14	3	2	36
No prior alkylator therapy	5	1	1	40
Prior alkylator therapy	9	2	1	33

TABLE 2.—Response by histologic type

Histologic type	No. of patients	No. of patients with—		Response rate (%)
		CR	PR	
Papillary serous cystadenocarcinoma	8	2	1	38
Mucinous cystadenocarcinoma	1	0	0	
Endometrioid	1	0	1	
Undifferentiated adenocarcinoma	3	0	0	
Granulosa cell	1	1	0	
Total	14	3	2	36

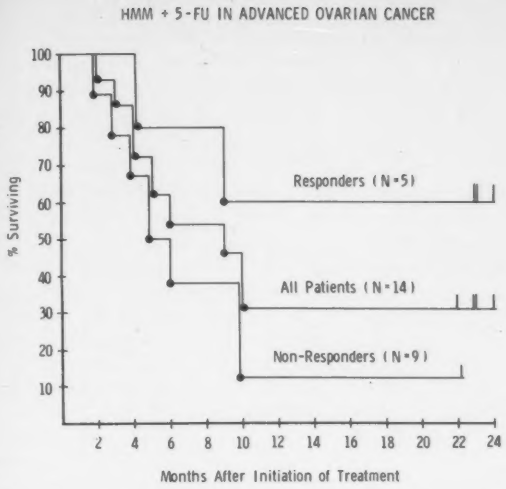

FIGURE 1.—% of patients surviving 0–24 mos after initiation of treatment with HMM and 5-FU for advanced ovarian carcinoma. There were 14 evaluable patients, 9 nonresponders and 5 responders.

two treatment cycles, maximal response did not occur until they had received three, four, or even five cycles of therapy. The fact that five of 14 patients had to have treatment discontinued because of toxicity after one or two cycles may imply that these patients were not able to tolerate this treatment and never received an adequate trial.

The response durations of the two partial responders were brief; one was 2 months and the other was 3 months. Both of the partial responders have died of their disease. The response durations of the three complete responders have been prolonged. All three of the complete responders are alive and free of disease at 23+, 23+, and 24+ months. Two of the three complete responders had had prior alkylator therapy. The survival of all patients, responders, and nonresponders is displayed in figure 1. The median survival time from the initiation of treatment for all patients is 9 months. The median survival for the nonresponders is 5 months. But, the median survival of the responding group has not yet been reached and is now > 24 months.

DISCUSSION

The combination of HMM and 5-FU evaluated in this WCSG pilot study is active in advanced ovarian carcinoma. However, the overall response rate of 36% is not greater than would be expected for either HMM or 5-FU alone; however, response durations and survival of complete responders were gratifyingly long (24+ months).

The toxicity of this regimen was excessive. The dominant toxic effects were GI, but unpredictable hematopoietic and neurotoxic effects were also observed. In all of the responders a 50% dose reduction of both agents was necessary after one or two treatment cycles. It therefore seems that a 50% dose level may have been adequate.

It is possible that a basic premise of combination chemotherapy was violated during this study, ie, HMM may act as an antipyrimidine and 5-FU is a known antipyrimidine. The use of two antipyrimidines in combination may have resulted in the unpredictable hematopoietic toxicity, the apparent lack of enhancement of therapeutic effect, and the response to 50% dose levels.

There appears to be a subset of patients who develop tolerance to the GI toxicity, as well as a subset of patients who achieve prolonged CRs to the combination of HMM and 5-FU. The combination is active in alkylator failures. However, it is anticipated that this combination will have limited usefulness because of the apparent lack of additive therapeutic effect and the excessive GI and unpredictable hematopoietic toxic effects.

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Enhancement of Bone Marrow Toxicity of VM-26-CCNU Combination by Bleomycin [1,2]

J. Hildebrand,* D. Gangji, C. Nouwynck, and D. Machin [3]

Combinations of drugs in cancer chemotherapy aim to associate substances which differ by their mechanisms of action and their side effects. Another reason for drug association is the potentiation of drug activities. Theoretically, changes in cell kinetics may lead to such potentiation. Pouillart et al (1) advocated this mechanism to explain the potentiation of CCNU effects by VM-26 observed by them in treatments of several tumors including human brain tumors.

The purpose of this phase I study was to test the toxicity of the combination VM-26, CCNU, and bleomycin (BLM) in patients with malignant brain tumors receiving radiation therapy. BLM was added to VM-26 and CCNU for the following reasons: (*a*) this drug has been given to patients with malignant brain tumors by the intraventricular and iv routes and occasional responses were observed (2); (*b*) BLM penetrates into brain tumors, especially the metastatic ones (3); and (*c*) BLM is nontoxic for the bone marrow when used alone.

This paper reports an unexpected and severe toxic effect, wbc count depression, observed in patients treated with VM-26, CCNU, and BLM. Such toxicity was not present in a similar group of patients treated with only VM-26 plus CCNU.

MATERIALS AND METHODS

Twenty-six patients (table 1) with an adequately assessed diagnosis of primary or secondary brain tumors were treated with VM-26, CCNU, and BLM (group I, ten patients) or with VM-26 and CCNU (group II, 16 patients). VM-26 was administered iv on Day 1 at a dose of 100 mg/m^2 as a 1-hour infusion. CCNU was given orally at a dose of 130 mg/m^2 on Day 2. The administration of these two drugs was repeated every 6 weeks. BLM (given only to patients in group I) by iv push at a dose of 20 mg/m^2 (maximum weekly dose, 30 mg) was administered every week starting on Day 2 (maximum total dose, 200 mg/m^2). BLM was given only once to two of ten patients; the remaining eight patients received three to six doses (mean, 4.3) during the first cycle. Concomitantly to chemotherapy all patients received brain irradiation of 6000 rads for primary tumors and 4500 rads for metastatic tumors. The weekly dose was similar (1000 rads) for the two types of tumors. Dexamethasone was given as required.

The patients were not allocated by randomization but the two groups appeared retrospectively to be comparable with respect to age and prior chemotherapy. However, group I had a higher proportion of metastatic tumors, and the male to female ratio was higher in group II (table 1).

Prior to the study, wbc and platelet counts were normal, and hemoglobin values were > 11 g/100 ml in all patients. Bone marrow, examined in 12 patients (six in each group), showed normal cellularity and there was an absence of neoplastic cells in all cases.

Previous chemotherapy given only to five of 26 patients had been interrupted ≥ 5 weeks before the present treatment.

TABLE 1.—Patient characteristics

Characteristic	Group I (VM-26, CCNU, BLM)	Group II (VM-26, CCNU)
Total No. of patients	10	16
Mean age in yrs (range)	58.4 (44–72)	59.6 (40–73)
Men	3	9
Women	7	7
No. of patients with—		
Primary brain tumor	1	11
Metastatic brain tumor	9	5
Previous chemotherapy	3	2
Bone marrow examination	6	6

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The wbc and platelet counts and the hemoglobin level were determined at least twice a week; liver function tests and BUN levels were obtained once a week.

The statistical analysis of the wbc counts was made in the following way. It is widely accepted that a wbc count of $< 3000/mm^3$ represents a serious toxic manifestation in patients. From the data collected, it was therefore possible to measure for each patient the number of days, calculated from the day of first administration of the chemotherapy, until the patient first experiences this level of toxicity. The resulting "toxicity-free" intervals for the two groups (ie, with and without BLM) can then be compared using the Mantel-Haenszel test (4,5).

A similar analysis using toxic levels of 60,000 platelets/mm^3 and 10 g/100 ml for the hemoglobin was also made.

RESULTS

The effects of each chemotherapy regimen (VM-26 and CCNU versus VM-26, CCNU, and BLM) on the wbc and platelet counts and hemoglobin level during the first 6 weeks of treatment are compared in figure 1.

The most striking finding was the difference in the wbc count (fig 1a). Thus, in group I there was an early drop in the leukocyte count to a mean geometric value of $1500/mm^3$, occurring around Day 10. In eight of ten patients in group I the wbc count

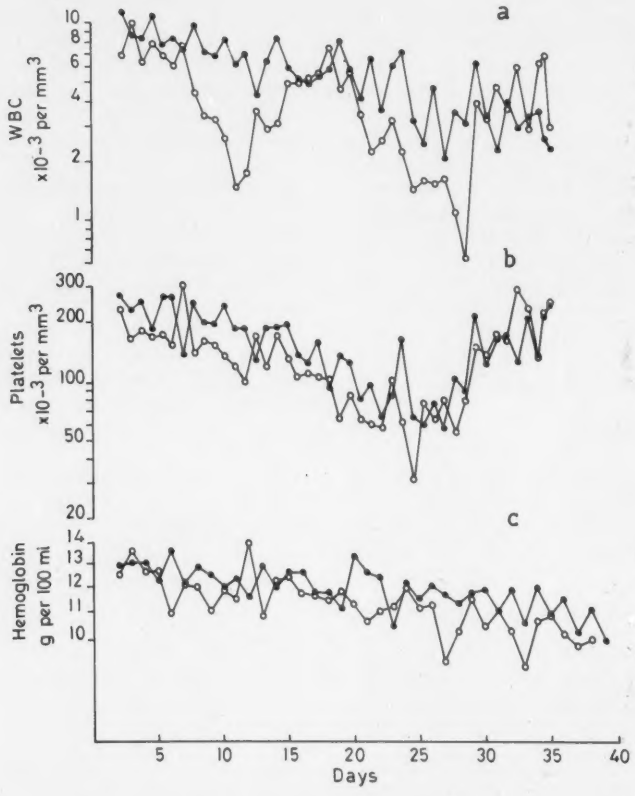

FIGURE 1.—Changes in wbc (*a*) and platelet (*b*) counts and hemoglobin (*c*) levels during a 6-wk treatment with VM-26 + CCNU (●) or VM-26 + BLM and CCNU (○). Each point is a geometric mean of approximately 4 observations, the average No. of determinations for each patient being 14.

TABLE 2.—Median toxicity-free intervals

Treatment group	Total No. of patients	No. of patients								
		Wbc count			Platelet count			Hemoglobin level		
		q_l*	Median	q_u*	q_l	Median	q_u	q_l	Median	q_u
I	10	8	12	21	21	24	67	11	19	23
II	16	28	33	39	24	31	42	24	38	46

*q_l = lower quartile; q_u = upper quartile.

was < 2000/mm^3 and in five patients the total granulocyte count fell to < 1000/mm^3; the wbc count remained > 3000/mm^3 in only one of ten patients. This early depression of the wbc count was not observed in group II where wbc counts remained at >3000/mm^3 in all patients during the first 18 days of treatment. The early drop in the wbc count was observed in the two patients who received only one dose of BLM, and such drops were no more pronounced in the three patients in group I who received prior chemotherapy as compared to patients without previous treatment. During the fifth week after onset of chemotherapy the wbc count was again depressed in both groups. However, the nadir was more pronounced in patients receiving BLM in addition to VM-26 and CCNU. Wbc counts <3000/mm^3 were found in six of eight patients in group I but in only three of 14 patients in group II during the same period.

The results of the statistical analysis are given in table 2.

The Mantel-Haenszel test for comparison of the toxicity-free first intervals for wbc and platelet counts and hemoglobin levels gave chi-squared values with 1 degree of freedom of 7.36, 0.01, and 8.12, respectively, indicating no differences in the platelet toxicity between the groups but significantly earlier changes in the wbc count ($P < 0.01$) and hemoglobin level ($P < 0.005$).

It is evident from table 2 and figure 1 that the differences in median toxicity-free first interval are the result of the steep drop in wbc count during the second week of treatment (median, 12 days) of group I patients. The greater toxicity with respect to the hemoglobin level in this group did not become apparent until about a week later (median, 19 days). There appears to be no significant difference in changes in the platelet count between the two groups; toxicity occurs during the fourth and fifth weeks (median, 28 days). Septicemia appeared in two patients whose granulocyte count fell to < 1000/mm^3. Both patients responded to antibiotic combination treatment. No death was attributed to the observed toxicity.

DISCUSSION

Severe leukopenia was observed between Day 8 and 13 of the treatment only in the group of patients receiving BLM in addition to VM-26 and CCNU. This early wbc count depression can not be attributed to differences in radiation therapy, age, or previous chemotherapy. Indeed, the two groups received the same daily doses of cranial irradiation before and during the early drop of wbc counts, and the mean ages and number of patients who received previous chemotherapy were comparable in the two groups.

A possible cause for an increased bone marrow sensitivity in the group of patients receiving BLM is the much higher proportion of metastatic brain tumors. Indeed, extraneural primary neoplasms invade the bone marrow much more frequently than primary brain tumors. It is unlikely however that bone marrow metastases account for the wbc count depression observed in the group receiving BLM, since six of ten patients had normal bone marrow examinations and two others had normal roentgenograms and isotopic scans of the skeleton. In addition, neoplastic infiltration of the bone marrow would not explain why specific changes occurred for wbcs and hemoglobin but not for platelets.

It is also unlikely that previous chemotherapy accounts for the hematologic toxicity in group I, since only three patients in this group had received antineoplastic drugs prior to the present trial and the blood toxic effects were not more severe in these three patients as compared with seven others in group I. Two patients in group II who had previous chemotherapy showed no sign of early hematologic side effects.

In the absence of any evident explanation, we assume that the addition of BLM to the VM-26-CCNU combination causes the early hematologic toxicity. The drop in wbc count particularly occurs after two or even one administration of 20 mg/m^2 of BLM. Given alone, such low doses of BLM are unable to cause blood toxic effects. One has then to

postulate a synergistic toxic effect of the VM-26-BLM-CCNU combination on the bone marrow. The mechanism of this toxic effect has not been elucidated.

Although the leukopenia was reversible in all cases, its high frequency and severity have justified the discontinuation of the VM-26-BLM-CCNU combination as used in this trial. Although the decrease in hemoglobin levels is greater in patients receiving BLM, this appears to have little biologic and clinical relevance. On the other hand, we have no evidence from the present study that the addition of BLM increases the antineoplastic activity of the VM-26-CCNU combination in secondary or primary brain tumors.

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Chemotherapy for Metastatic Carcinoid Tumors: Experiences With 32 Patients and a Review of the Literature [1,2]

Sewa S. Legha,* Manuel Valdivieso, Robert S. Nelson, Robert S. Benjamin, and Gerald P. Bodey [3]

SUMMARY

An analysis of results of chemotherapy in 32 patients with metastatic carcinoid tumors was carried out. The most frequently used chemotherapeutic agent was 5-fluorouracil (5-FU) followed by nitrosoureas in combination with 5-FU or with cyclophosphamide. Adriamycin (used as adriamycin-DNA complex) alone or in combination with other drugs was used in seven patients. Seven partial responses were observed, five of them occurring in the patients receiving adriamycin-containing regimens. Based on these findings, adriamycin deserves further evaluation in metastatic carcinoid tumors.

[Cancer Treat Rep 61:1699–1703, 1977]

Carcinoid tumors arise in the gastrointestinal tract, biliary tree, pancreas, lung, and ovaries. Because primary tumors are generally asymptomatic, most carcinoid tumors are metastatic at the time of clinical diagnosis. Metastatic tumors originating in foregut and midgut structures are often functional and produce symptoms of the carcinoid syndrome, whereas rectal carcinoids are nonfunctional and pose problems which are related to metastatic tumors in general. Even with widespread metastases some carcinoid tumors grow slowly and patients can live for years without much disability. Other patients do not fare as well, and the tumor progressively grows and proves fatal in a relatively short period of time. The reported median survival of patients with metastatic carcinoid is 2 years and approximately 80% of such patients are dead within 5 years of diagnosis (1).

The current treatment approach to metastatic carcinoid tumors is far from satisfactory and needs refinement. In contrast to the extensive work on the mechanisms and control of the carcinoid syndrome, drug treatment of the underlying malignancy has received little attention in the literature. Information on chemotherapy for malignant carcinoid tumors is presently quite limited. Therefore, we have reviewed our experiences with patients referred to the M. D. Anderson Hospital and Tumor Institute for the treatment of symptomatic carcinoid tumors. The results of our treatments using a variety of chemotherapeutic agents are presented, and our findings in the light of experiences reported by other investigators are discussed.

MATERIALS AND METHODS

We made a search for patients with carcinoid tumors of all sites treated with chemotherapy at the M. D. Anderson Hospital and Tumor Institute through the years 1944–1976. Patients were included in this analysis if they had received cytotoxic agents at any time after the diagnosis of metastatic carcinoid. Most patients were referred to our institution for treatment after their disease was diagnosed as carcinoid. The referring physician's diagnosis had to be confirmed by our pathologists by a review of slides and tissue blocks, or sometimes by a second biopsy done at our hospital. In cases of equivocal diagnosis, confirmation was made by electron microscopic examination. Most patients had a complete workup with blood cell counts, serum chemistries, and measurement of 24-hour 5-hydroxyindoleacetic

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acid (5-HIAA) excretion in the urine. A search for primary tumors was made with barium study of the gastrointestinal tract, proctosigmoidoscopy, and chest roentgenograms. Most patients had liver scans and some had abdominal ultrasound examinations, angiographies, and occasionally, bilateral lower extremity lymphangiograms.

The chemotherapeutic agents utilized varied according to their availability and to the choice of physicians treating individual patients. Most patients received systemic chemotherapy, but three patients were initially given one or two infusions of 5-fluorouracil (5-FU) into the hepatic artery and subsequently were given iv 5-FU as maintenance. The intensity of drug therapy varied, but most patients received treatment to their limits of tolerance. The duration of chemotherapy was also determined by patient tolerance, as well as therapeutic benefit. Response to chemotherapy was determined by measurement of urinary 5-HIAA, or by changes in the tumor size measured by physical examination, roentgenographic studies, or radioisotopic liver scans. A partial response (PR) is defined as $\geq 50\%$ reduction in the sum of the products of two perpendicular diameters of measurable tumor lesions without appearance of new lesions. If hepatomegaly was the primary indicator of response, there must have been a reduction of the sum of liver measurements below each costal margin at the mid-clavicular lines and xiphoid process by at least 30%, accompanied by improvement in liver functions and liver scan. Changes in urinary 5-HIAA alone unaccompanied by changes in tumor size or symptoms were recorded but not considered in response evaluation. Total regression of all visible tumor was called a complete response (CR). Patients with $< 50\%$ tumor regression or with improvement in symptoms accompanied by no change in tumor size for at least 6 months were classified as having stable disease. Patients who had no symptomatic benefit, continued increase in tumor size and 5-HIAA excretion, or appearance of new lesions were classified as having progressive disease.

RESULTS

A total of 128 patients with carcinoid tumors have been seen at our institute during the past 32 years. No chemotherapy was utilized in the years prior to 1961.

Between 1961 and 1976, 11 men and 21 women with metastatic carcinoid received 38 trials of chemotherapy (table 1). The indications for chemotherapy included symptomatic metastases, such as

TABLE 1.—Chemotherapy of carcinoid tumors—M. D. Anderson Hospital experience

Regimen	No. of patients	No. of responses	
		PRs*	Stable disease
5-FU†	14	—	1
Cyclophosphamide + methyl-CCNU	4	—	1
Cyclophosphamide	3	—	—
5-FU + cyclophosphamide + CCNU	2	—	1
Ftorafur + adriamycin + BCNU	2	1(52+)	1
Adriamycin + 5-FU	3	2(52, 44)	1
Adriamycin-DNA complex	2	2(24, 24)	—
Neocarzinostatin	2	—	—
Streptozotocin	1	—	—
Bleomycin-COMF‡	1	1(12)	—
Ftorafur + methotrexate + methyl-CCNU	1	—	—
Piperazinedione	1	—	1
Pyrazofurin	1	—	—
Cyclophosphamide + methotrexate	1	1(12+)	—

*Nos. in parentheses = duration of PR in wks.

†3 patients initially received 5-FU by hepatic artery infusion but later received iv 5-FU maintenance therapy.

‡COMF = cyclophosphamide, vincristine (Oncovin), methotrexate, and 5-FU.

massive hepatomegaly with or without symptoms of carcinoid syndrome, or extra-abdominal metastases. The median age for the group was 56 years (range, 36–71 years). Twelve patients were ≤ 50 years old. The site of primary tumor was as follows: small intestine, nine patients; rectum, six; colon, five; appendix, three; bronchus, two; stomach, one; mediastinum, one; and unknown primary, five. Metastatic tumor involved the liver in 24 patients, supraclavicular lymph nodes in five, lungs in three, bones in two, skin in two, and breast in two.

For the purpose of analyzing responses to treatment, we have arbitrarily divided the patients into two groups. A group of 21 patients who received at least two courses of chemotherapy and were alive for ≥ 3 months following the treatment are hereafter classified as patients who had an adequate trial. There were 17 single chemotherapeutic trials in this group, two therapy changes for three patients, and one patient had three treatment changes. Many patients were treated with combinations of chemotherapeutic agents that were under investigation for other gastrointestinal tumors. 5-FU was the most commonly used single agent. It was received by seven patients and was commonly used in an initial loading course of daily treatments followed by weekly maintenance therapy. Some patients were treated with phase I drugs either initially or subsequent to other regimens.

The second group had 11 patients who received chemotherapy for short periods. Ten of the 11 patients survived for < 3 months after the beginning of chemotherapy, making it doubtful if chemotherapy had any therapeutic benefit in this group. Most of these patients were treated prior to 1970 and received single-agent chemotherapy. Thus, 5-FU was used for seven patients (five iv and two intra-arterial), cyclophosphamide for three, and a combination of cyclophosphamide and methotrexate for one.

The therapeutic effects of chemotherapy are summarized in table 1. Twenty-five of the 38 treatment trials were without therapeutic benefit. There were seven patients with PRs and six patients with stable disease. Six of the PRs were observed in patients with nonfunctional carcinoids; three had a primary tumor in the rectum, one in the mediastinum, one in the bronchus, and one in an unknown site. There was only one PR among 12 patients with the carcinoid syndrome. The sites of response included three examples of massive hepatomegaly, two patients with multiple pulmonary and one with pleural and pulmonary metastases, and one patient with pelvic and supraclavicular masses. The chemotherapy used for the responders included adriamycin-DNA complex in two, adriamycin + 5-FU in two, adriamycin + ftorafur + BCNU in one, cyclophosphamide + methotrexate in one, and bleomycin + cyclophosphamide + vincristine + methotrexate + 5-FU in one patient. The median duration of PRs was 24 weeks (range, 12–52+ weeks). Although stabilization of disease as an effect of chemotherapy in carcinoid tumors is difficult to interpret, stable disease in six patients lasted from 6 months to 1 year.

The effects of chemotherapy on 5-HIAA excretion were quite variable. As mentioned earlier, only one of the partial responders had a functional carcinoid. This patient had an initial 5-HIAA level of 28 mg/24 hours, and had amelioration of her symptoms and stabilization of her hepatomegaly for 1 year. No followup 5-HIAA level was determined. She then developed bilateral pulmonary metastases which regressed after she was given a different combination of drugs. Among the six patients with stable disease (all with carcinoid syndrome) the 5-HIAA level decreased in one, stayed stable for 1 year in one, and increased in two in spite of symptomatic relief and regression in liver size. Two patients had no followup determinations.

The median survival of 21 patients in the adequately treated group was 24+ months (range, 4–84 months). Fourteen of these patients were alive at the beginning of 1977, but only seven of them are currently being followed and are receiving chemotherapy. Seven patients were lost to followup.

DISCUSSION

Because of the rarity of metastatic malignant carcinoids, experience with cancer chemotherapeutic agents for the treatment of patients with this problem has been relatively sparse. In many patients the disease may remain indolent without the need of antitumor drugs. However, there are patients with more aggressive tumor growth who will present with progressive liver metastases, symptoms of partial bowel obstruction, ascites, or severe uncontrollable carcinoid syndrome for whom antitumor therapy will be a consideration. Although antihistamines and antiserotonin compounds, such as methysergide, have been used to control flushing and diarrhea of carcinoid syndrome, their effects vary and they rarely provide complete symptomatic relief (2). The average age of patients with carcinoid tumors at the time of their diagnosis is 56 years, which is approximately 10 years younger than the average age of patients with noncarcinoid neoplasms arising in the same organs (3,4). The relative youth and uniform fatal course of the disease (average survival, 2 years following liver metastases), justifies treatment in all patients especially

TABLE 2.—Reported experience with chemotherapy of carcinoid tumors

Treatment	No. of patients	Response: No. of PRs (%)	Response: Duration (wks)	Ref No.
Single agents				
Alkylating agents	33	9(27)	—	5–9
Streptozotocin	17	5(30)	16	10–12
5-FU	15	6(40)	16	5
Actinomycin D	9	1(11)	—	5
Methotrexate	6	1(16)	—	7
Mitomycin C	3	0	—	5
Adriamycin	1	1	—	13
Combination chemotherapy				
Cyclophosphamide + methotrexate	11	6(55)	—	7
5-FU + streptozotocin	9	6(66)	—	5
5-FU + BCNU	2	0	—	5
Cyclophosphamide + methotrexate + adriamycin	1	1	24	13

if they have significant symptoms and evidence of progressive disease.

The current knowledge of the effectiveness of chemotherapy in this type of tumor is quite limited (table 2). Although there are reports of antitumor activity with 5-FU (1,5) and alkylating agents (6–9), in general the responses have been brief and less than clear cut. Among the alkylating agents, cyclophosphamide and melphalan have shown some degree of activity (four of 16 and three of 15 responses, respectively). Streptozotocin is the only other single agent that has been used for a significant number of patients. Although initially reported to possess good activity by Moertel et al (10) and by Feldman et al (11), a later report by Schein et al was discouraging (12). We could find no reported data on the antitumor activity of the many other clinically used chemotherapeutic agents.

The data on combination chemotherapy are even more sparse. Mengel and Shaffer have claimed substantially higher response rates when cyclophosphamide and methotrexate are used together, compared to their activity when used alone (7). Similar claims have been made with a combination of 5-FU and streptozotocin as reported by Moertel et al (5). Based on these rather encouraging leads, the Eastern Cooperative Oncology Group is currently investigating combinations of streptozotocin and cyclophosphamide in comparison with streptozotocin and 5-FU.

The responses reported in the literature generally have been based on a variable and sometimes insignificant reduction of liver size and, therefore, may not have been substantial. The brief duration of these responses (average, 12–16 weeks) is a further indication of their actual magnitude.

The low response rate to fairly intensive chemotherapy regimens administered to the patients in this study indicates that carcinoids are relatively insensitive to many of the available antineoplastic agents. In this regard the lack of benefit from the use of 5-FU and the nitrosoureas was particularly surprising (table 1). The lack of effectiveness of nitrosoureas shows that carcinoid tumors differ somewhat from other gastrointestinal cancers in their responsiveness to chemotherapy. A similar fact of considerable significance relates to the responses that were seen with adriamycin (used as adriamycin-DNA complex) or combinations containing this agent. Antitumor activity of adriamycin against this tumor has also been reported in a recent article (11). The majority of the significant responses occurred in nonfunctioning tumors, most of which originated in the rectum. Only one of the patients with carcinoid syndrome showed objective tumor regression, although some showed symptomatic improvement.

Based on this analysis we are continuing the investigation of adriamycin and other anthracycline antibiotics administered alone or in combination

with other agents in the treatment of patients with symptomatic carcinoid tumors at this institution.

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Phase I Clinical Study of Carminomycin: Its Activity Against Soft Tissue Sarcomas [1]

Brief Reports and Preliminary Communications

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One of the most interesting problems in cancer therapy in recent years is the determination of differences in the biologic activity of various anthracycline antibiotics. Further interest in this group of compounds has been generated by a new drug isolated in the USSR, carminomycin. Preliminary experiments have disclosed a high degree of activity against experimental tumors as well as pharmacologic differences in comparison to adriamycin and daunorubicin (1–7).

Preliminary experiments in mice demonstrated that carminomycin was well absorbed, as indicated by the close correlation of LD50 doses when given by ip or oral administration. The LD50 of carminomycin for these mice was 3.7 mg/kg when given parenterally compared to 7.3 mg/kg when given orally. Equitoxic doses of carminomycin administered orally and iv were equally effective against several rodent leukemias, the Lio-I lymphosarcoma and Sarcoma 180, but oral administration was more effective against the ascitic form of NK/LI leukemia.

The primary toxicity of carminomycin in dogs is hematopoietic, with doses of 0.15 mg/kg/day iv × 10 days producing lethal bone marrow suppression. There appeared to be less cumulative toxicity from carminomycin than daunorubicin or adriamycin when these three drugs were assessed in hybrid mice.

Only limited pharmacokinetic information regarding carminomycin is available in animals. In rabbits, the drug appears to be highly protein bound, 70%–90%. Drug levels were detectable in plasma for 5 hours while concentrations were present in spleen for up to 24 hours. Biliary elimination predominated over urinary excretion.

Preliminary clinical studies of carminomycin demonstrated activity against soft tissue sarcoma, acute leukemia, lymphomas, carcinoma of the breast, and other tumors (9–12). We present here the findings of phase I trials as well as phase II studies in patients with soft tissue sarcomas.

MATERIALS AND METHODS

Carminomycin hydrochloride was supplied in 5-mg ampules by the New Antibiotics Research Institute of the USSR Academy of Sciences. It was dissolved in physiologic saline and administered iv. Care was taken to insure that the iv line was patent since the drug causes subcutaneous tissue necrosis when infiltrated. Three-week cycles of treatment were evaluated in the phase I trial, and there were 78 patients in the study. Repeat courses of the treatment were carried out every 3–4 weeks, and doses were reduced by 30% in instances where hematologic toxicity resulted from previous courses.

RESULTS

The dose escalation used is shown in table 1. Single doses of 4–8.5 mg/m² were given twice per week (every third day) for 3-week courses. The total dose for these courses varied from 27 to 40 mg/m². A total of 78 patients were treated in this phase of the study. With this schedule, doses of approximately 8.5–10 mg/m² were tolerated poorly; 55% of patients so treated had vomiting. Leukopenia (< 4000 cells/mm³) was observed in 60% of patients at this dose level while 58% of patients receiving approximately 6 mg/m² had leukopenia when these doses were given on a twice weekly basis. Minor side effects of both dose levels included headaches,

TABLE 1.—Dose escalation plan for phase I study of carminomycin*

No. of patients	Single dose (mg/m²)	Total dose/course (mg/m²)
8	4	27
35	6	35
4	7	40
20	8.5	40
11	6–8.5	33

*All doses were given twice a wk for 3 wks, with a rest period of 3–4 wks between courses.

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TABLE 2.—Leukopenia following carminomycin

Dose (mg/m²)	No. of patients with leukopenia/No. treated (%)	No. of patients with wbc count nadir (cells/mm³)—			
		< 1000	1001–2000	2001–3000	3001–4000
6	20/35 (57)	1	2	6	11
8.5	12/20 (60)	4	5	2	1
		Mean duration of leukopenia (days)			
		14.0	11.7	9.6	4.1

TABLE 3.—Leukopenia during 5-day carminomycin regimen (5 mg/m²/day)

Course	No. of patients with leukopenia/No. treated (%)	No. of patients with wbc count nadir (cells/mm³)—			
		< 1000	1001–2000	2001–3000	3001–4000
1	18/18 (100)	1	5	3	9
2	14/14 (100)	—	5	3	6
3	3/3 (100)	—	1	1	1

TABLE 4.—Carminomycin for soft tissue sarcomas

Histologic type	No. of patients	No. of patients with—	
		> 50% response	< 50% response
Synovial cell sarcoma	6	3	1
Leiomyosarcoma	15	2	4
Rhabdomyosarcoma	7	2	1
Stromal sarcoma of uterus	7	3	2
Liposarcoma	2	1	—
Fibrosarcoma	3	1	—
Angiosarcoma	6	1	1
Unspecified	2	—	—
Total	48	13 (27%)	9 (19%)

general weakness, anorexia, alopecia, stomatitis, and rarely, diarrhea.

Table 2 shows the relationship between dose of carminomycin and leukopenia. It can be seen that more severe degrees of leukopenia were associated with the 8.5-mg/m² dose given twice weekly. The period of leukopenia with this schedule lasted from 4 to 14 days. Patients had less platelet production toxicity, with < 30% of patients treated at the 6–8.5-mg/m² twice weekly schedule having platelet counts < 150,000/mm³.

Repeat courses of therapy given at 1-month intervals gradually led to the necessity of a 30% reduction in the dose for subsequent courses. No clinical symptoms of cardiotoxicity from carminomycin have been detected thus far. Four patients have had tachycardia of undefined character, and six patients have had transitory changes on their electrocardiograms such as lowering of T waves in chest leads. The significance of these changes is unclear. There has been no evidence of toxicity for the liver, kidneys, or other organs using the twice weekly schedule for 3 weeks.

In addition to the twice weekly schedule, a 5-day course of treatment at doses ranging between 1.5 and 5 mg/m²/day was evaluated in 13 patients. A 5-day course at 5 mg/m²/day led to leukopenia in six of seven patients. The 5-day regimen at 5 mg/m² was evaluated in 18 patients with metastatic breast cancer. Fourteen of these patients received a second course of treatment and three received three courses. In the second course of treatment, the dose was reduced to 2–3 mg/m² for 5 days in five of the 14 patients, and a similar reduction was made in the third course of treatment in two of three patients. A 21–30-day interval was used between treatment courses. Severe leukopenia was the primary complication of treatment in these patients (as shown in table 3) since all patients showed a reduction in wbc count to < 4000/mm³. Leukopenia usually developed on the tenth day after beginning treatment and lasted for up to 25 days. In three patients thrombocytopenia also occurred after the first course of treatment.

The effectiveness of carminomycin against soft tissue sarcomas was evaluated in 48 adult patients, 43 of whom received the twice weekly regimen for 3

weeks and five of whom received the 5-day regimen. Seven of these patients had been previously treated with chemotherapy. Results are given in table 4. Fifty percent of the patients showed evidence of hematopoietic toxicity. Of 24 patients receiving two or more courses of treatment, 15 showed clinical improvement. The median duration of remission for those patients showing a $\geq 50\%$ decrease in tumor size was 9 months, while those showing $< 50\%$ improvement had a median duration of remission of only 3.5 months.

In conclusion, the clinical trial of carminomycin has shown a considerable degree of antineoplastic activity in soft tissue sarcomas. The primary toxic effect has been leukopenia with lesser thrombocytopenia. There has been no evidence of cardiotoxicity in the patients we treated. Phase II and III studies are currently in progress.

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Treatment of Metastatic Melanoma With Combined 5-Fluorouracil and Procarbazine [1]

Brief Reports and Preliminary Communications

E. M. Nordman* and M. Mäntylä [2]

The treatment of disseminated melanoma with combined cytostatic agents has been practiced in recent years. Carter (1) has proposed the following guidelines for combining different cytostatic agents: (*a*) each drug should be active when used alone against the neoplasm in question, (*b*) the drugs should have different mechanisms of action, and (*c*) the toxic effects should not overlap so that each drug can be given at a dose level near its maximum tolerated dose.

5-Fluorouracil (5-FU) inhibits the synthesis of RNA and by reacting with thymidylate synthetase interferes with the formation of DNA (2).

The action of procarbazine (generously supplied by Hoffmann-La Roche and Co, Ltd) resembles the effect of alkylating agents. Procarbazine produces depolymerization of the DNA molecule in the presence of oxygen (3). Brulé et al (4) first used procarbazine in the treatment of solid tumors. Nordman (5) reported a favorable response in seven of 16 patients with combined 5-FU and procarbazine.

In 1967, treatment of disseminated melanoma with this drug combination was initiated in the radiotherapy department of the Middle Finland Central Hospital.

MATERIALS AND METHODS

Nineteen patients with disseminated melanoma received combined treatment with 5-FU and procarbazine from 1967 to 1975. Eight of these patients were men and 11 were women. The ages ranged from 21 to 66 years (mean, 51 years). Eleven of the patients had their principal metastases in the lungs, two in the liver, one in the brain, four in the skin or subcutis, and one in the lymph nodes. In all cases the primary tumor was verified histologically.

The 5-FU treatment was given during the first 4 days with doses of 10–15 mg/kg as daily infusions in 5% glucose, each infusion lasting 3–4 hours. 5-FU was continued at a dose of 7.5 mg/kg on Days 6, 8, 10, 12, etc, unless toxicity occurred. Procarbazine, 250 mg, was added to each infusion bottle. The total dose was 6–10 g of 5-FU and 2–4 g of procarbazine during a 2–4-week period. The cytostatic course was repeated at intervals of 6–12 weeks. The actual metastases were measured during and after the treatment, and the general condition and the blood values were checked at least once a week. All patients were followed until death, which occurred within 1–22 months after the beginning of therapy.

The results were classified as complete response (complete disappearance of metastases lasting for at least 1 month), partial response (> 50% reduction in pretreatment measurements lasting for 1 month), stabilized disease (growth of the metastases retarded for at least 1 month), and no response (metastases continuing to grow).

RESULTS

The pulmonary metastases disappeared totally in two patients for 4 and 5 months after treatment. In five cases the metastases diminished by at least 50% for at least 2–6 months. In three cases the growth of the metastases was retarded. Nine patients did not show any effect of therapy (table 1).

The median survival was 7.5 months for patients showing a complete response, 8 months for patients with a partial response, 3 months for patients with no change in metastases, and 3 months for patients with no response.

The toxic effects of the 5-FU and procarbazine combination were nausea and vomiting in ten patients and leukopenia in one patient. On three occasions the course of treatment had to be terminated because of heavy vomiting.

DISCUSSION

Of the 19 patients with disseminated melanoma, eight (42%) demonstrated complete or partial response to treatment with combined 5-FU and procarbazine. Additionally, in three patients the disease was stabilized. The median survival of these 11 patients was 9 months, considerably longer than the 3-month survival of patients with no response.

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TABLE 1.—Response according to location of metastases in melanoma patients treated with 5-FU and procarbazine

Location of metastasis	No. of patients with—			
	Complete response	Partial response	Stabilized disease	No response
Lung	2	3	1	5
Liver			1	1
Brain				1
Skin and subcutis		1	1	2
Lymph nodes		1		
Totals	2	5	3	9

Luce (6) has reported that procarbazine is one of the most effective single therapeutic agents in disseminated melanoma, with 28% of patients responding to this drug alone, and he has also stated that some cytostatic agents not effective alone may be effective in combinations.

Until now, procarbazine has not been included in the usual chemotherapy schedules used in disseminated melanoma. In the report of Luce (7), procarbazine did not enhance the effect of DTIC as compared with DTIC alone, the drug most often included in melanoma therapy. Procarbazine did not even enhance the effect of cyclophosphamide and vincristine in melanoma according to a recent report of Byrne (8).

The results achieved in this study indicate the necessity of further experiments with procarbazine in different combinations for the treatment of disseminated melanoma. The combination of procarbazine (inducing depolymerization of DNA) and 5-FU (interfering with nucleic acid synthesis) seems to be a rational one since these drugs affect different phases of the cell cycle.

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Phase II Evaluation of Piperazinedione in Metastatic Breast Carcinoma [1,2]

Brief Reports and Preliminary Communications

R. L. Palmer, B. A. Samal, C. B. Vaughn, and B. L. Tranum [3,*]

Piperazinedione, a crystalline antibiotic, has been found to be active in several murine tumors (1,2).[4] Its activity suggested that it may inhibit DNA synthesis and may act as an alkylating agent. Drugs with this spectrum of activity have often been found to be effective in the treatment of breast carcinoma. Phase I studies suggested that piperazinedione might be effective in breast cancer; no major toxicologic disadvantages other than myelosuppression were noted (3). In 1975, the SWOG began a phase II trial in patients with advanced metastatic carcinoma of the breast who had failed to respond to or had relapsed after conventional hormone therapy or chemotherapy.

MATERIALS AND METHODS

Fifty-eight patients with histologically proven breast cancer were entered into this study between June 26, 1975, and February 19, 1976. All patients had been exposed to multiple-drug chemotherapy and the majority were > 5 years postmenopausal. Seventy percent of the patients had osseous metastases and 40% had visceral metastases. All patients had progressive metastatic disease not thought to be responsive to more standard chemotherapeutic or hormonal approaches.

Pretreatment evaluation consisted of a cbc count including platelet determination, urinalysis, chest roentgenogram, BUN, serum creatinine, bilirubin, calcium, alkaline phosphatase, and SGOT. Isotope scans and additional roentgenographic studies were obtained when indicated.

Piperazinedione was supplied in lyophilized form in 5-mg vials. The drug was dissolved in 5 ml of sterile water and administered iv in at least 100 ml of physiologic saline over a time course of not < 20 minutes. The initial dose was 12 mg/m² administered at 3-week intervals. Twenty-nine patients were treated in this manner, but, because of severe thrombocytopenia, the dosage was reduced to 9 mg/m² every 3 weeks. Twenty-nine patients were treated at this dose level.

Blood cell counts were obtained every 3 weeks or more frequently if the nadir had not been reached prior to the next course. Subsequent courses of piperazinedione were adjusted in relation to the degree and duration of myelosuppression. The following dose levels were utilized for dose adjustments: 15, 12, 9, and 6 mg/m². Subsequent courses were delayed until the platelet count rose to 100,000/mm³ or the leukocyte count to 3000/mm³. An adequate trial of treatment for evaluability of patient response consisted of at least two courses of treatment. Early death was defined as a patient who had less than an adequate trial.

The criteria of response were defined as follows: complete remission, disappearance of all known metastases for a minimum of 4 weeks; partial remission, ≥ 50% decrease in the sum of the product of the diameters of the measured lesions for a minimum of 4 weeks with no simultaneous increase in any lesion or appearance of new lesions; stable disease, a stable state or a response < 50% and no further increase in tumor size for 4 weeks; and increasing disease, unequivocal increase in measured lesions or appearance of new lesions.

RESPONSES

Three of the 58 patients (5%) showed a partial therapeutic response. All responders had soft tissue metastases alone; no response lasted for > 10 weeks. Forty-two patients were fully evaluable. Of the 16 patients who were partially evaluable, 12 had an early death due to increasing malignancy and not drug toxicity, one patient was lost to followup, one

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[3] This study was conducted by the Southwest Oncology Group (SWOG). Scott and White Clinic, Temple, Tex (*R. L. Palmer*), Wayne State University, Detroit, Mich (*B. A. Samal*), Providence Hospital, Southfield, Mich (*C. B. Vaughn*), and University of Arkansas, Little Rock (*B. L. Tranum*).

[4] Preliminary data sheet: crystalline antibiotic pharmaceutical data. Prepared by the Developmental Therapeutics Program, Division of Cancer Treatment, NCI, Bethesda, Md, June 6, 1973.

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TABLE 1.—Piperazinedione toxicity for the 2 dose schedules

Dose (mg/m²)	No. of patients	No. of patients with—			
		Wbc nadir (cells/mm³)		Platelet nadir (platelets/mm³)	
		> 2000	< 2000	> 50,000	< 50,000
12	29	23	6	16	13
9	29	25	4	20	9

TABLE 2.—Piperazinedione: No. of courses and total drug dose with different treatment schedules

Dose (mg/m²)	No. of fully evaluable patients	Mean No. of courses/patient (range)	Mean total dose (mg)/patient (range)
12	19	2.4 (2–5)	40 (25–85)
9	23	2.8 (2–6)	34 (20–70)

refused further treatment, and in two patients, major protocol violations occurred. If these cases are deleted, a 7% response rate was seen (three of 42 patients).

TOXICITY

Myelosuppression was significant in both dose schedules (table 1). Piperazinedione at either dose level produced more severe thrombocytopenia than leukopenia. Forty-five percent of those patients treated with a dose of 12 mg/m² had thrombocytopenia with < 50,000 cells/mm³ while 30% developed similar thrombocytopenia at a dose level of 9 mg/m². The median time to the nadir of thrombocytopenia was 18 days (range, 11–80 days). The median time to the nadir of leukopenia was 14 days (range, 7–47 days). Thrombocytopenia persisted longer before recovery than did leukopenia, averaging 10 and 6 days, respectively. Table 2 demonstrates the mean number of courses and mean total drug administered to fully evaluable patients. Partially evaluable patients are omitted from this table since they had only two courses of treatment.

Nausea was present to a mild or moderate degree in 20% of the patients regardless of the dose schedule. There was no evidence of other drug-induced adverse reactions.

DISCUSSION

Piperazinedione was well tolerated by all patients except for nausea and vomiting and myelosuppression. Myelosuppression was significant, especially thrombocytopenia which frequently required delay in treatment or a dose reduction. The toxicity found in this study was similar to that observed in the phase I study (3).

Seventy percent of our patients had osseous metastases and this factor coupled with major prior polychemotherapy exposure may have played a significant role in predisposing our patients to myelosuppression. The recommended starting dose for patients with far-advanced malignancy who have undergone extensive prior chemotherapy is 9 mg/m² every 3 weeks. Patients with less extensive prior treatment may tolerate a dose of 12 mg/m². No appreciable antitumor effect was found in this group of patients. The three patients with soft tissue metastases who had a partial response exacerbated within 10 weeks; one was treated with 12 mg/m² of piperazinedione while the other two were treated with 9 mg/m².

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Central Nervous System Disturbances After Combined Administration of Procarbazine and Mechlorethamine [1,2]

Brief Reports and Preliminary Communications

Gary B. Weiss, Paul L. Weiden,* and E. Donnall Thomas [3]

Procarbazine and mechlorethamine are drugs commonly used in cancer chemotherapy and are commonly given together in low doses in a combination regimen used to treat Hodgkin's disease (mechlorethamine, vincristine, prednisone, and procarbazine [MOPP]). No drug interactions have been noted between procarbazine and mechlorethamine in the MOPP regimen. At high doses, however, procarbazine has been noted to cause somnolence (1) while mechlorethamine has been noted to cause central nervous system (CNS) stimulation (2). In the conditioning of leukemia patients for bone marrow transplantation, we routinely administer high-dose cyclophosphamide (CY) (120 mg/kg) followed by 1000 rads of total body irradiation (3). Occasionally in patients with pre-existing cardiac disease, high-dose mechlorethamine has been substituted for CY. High-dose procarbazine has been utilized before transplantation in patients previously exposed to family member blood products in order to lessen the likelihood of marrow graft rejection (4). In two patients, high-dose mechlorethamine and procarbazine were administered on the same day and both of these patients developed somnolence and disorientation lasting several days which are described in this report.

CASE REPORTS

Patient 1 (Unique Patient No. [UPN] 383)

A 21-year-old white man with acute myelogenous leukemia was admitted for marrow transplantation with advanced disease. There had been and was no evidence of CNS leukemia, and no cranial irradiation or chemotherapy had been given. Neurologic examination and lumbar puncture were normal on admission.

The patient's pretransplant regimen included procarbazine, 12.5 mg/kg, 8, 6, and 4 days before marrow transplantation, and mechlorethamine, 0.75 mg/kg, 4 and 2 days before transplantation (3). Three days before transplantation the patient was nauseated and lethargic but arousable and oriented. No additional sedatives or antiemetics were given. During the next 2 days he remained lethargic and was disoriented with no focal neurologic findings. On the day of bone marrow transplantation, the patient's confused mental status remained unchanged before and after 1000 rads of total body irradiation. Lumbar puncture on Day 1 after the marrow transplant demonstrated xanthochromic spinal fluid. By 3 days after the transplant the patient's mental status had improved. On Day 4 after the transplant an electroencephalogram showed generalized slow dysrhythmic activity arising from all areas over both sides of the brain suggesting a metabolic encephalopathy. The hospital course was complicated by sepsis, and the patient died 7 days after the transplant. An autopsy demonstrated focal, small petechial hemorrhages of the dura over the right frontal parietal region and thoraco-lumbar spinal cord.

Patient 2 (UPN 651)

A 22-year-old white man with acute myelomonocytic leukemia was admitted for marrow transplantation with advanced disease. Eight months before admission he had leukemic meningitis which was treated with intrathecal methotrexate and 3000 rads of cranial irradiation. At the time of admission, neurologic examination and lumbar puncture were within normal limits.

Eight, 6, and 4 days before bone marrow transplantation, he received 15 mg/kg of procarbazine in the morning. Four days prior to bone marrow transplantation, in the evening after premedication with 90 mg of phenobarbital, he received 1 mg/kg of mechlorethamine. That night he exhibited inappro-

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priate behavior at times and was talking in his sleep. In the morning he was disoriented and hallucinating periodically. Lumbar puncture was normal. For the next 3 days he was disoriented, lethargic, and incontinent. On the day of bone marrow transplantation he remained confused before and after 1000 rads of total body irradiation but had occasional lucid moments. An electroencephalogram 4 days after the bone marrow transplant was markedly abnormal with generalized diffuse, slow frequencies dominant at 1½–2 and 5–6/second, consistent with a severe diffuse organic process. A brain scan 6 days after transplantation was normal. The patient's mental status gradually improved, and he was last noted to be disoriented 8 days after the transplant. He died of recurrent leukemia 41 days following transplantation.

DISCUSSION

Both procarbazine and mechlorethamine are known to have CNS effects. The neurotoxic effects of procarbazine consist of changes in affect and disorders of consciousness (somnolence, depression, agitation, and psychosis) as well as peripheral neuropathies (1). These complications are dose-related. Chabner et al (5) found mild lethargy and somnolence in three of seven patients receiving 300–1000 mg/m^2. At higher doses (1500 mg/m^2), they noted somnolence for up to 72 hours with recovery characterized by lethargy, restlessness, apprehension, and emotional lability in seven of ten patients.

We have administered 12.5–15 mg/kg (approximately 500–600 mg/m^2) of procarbazine to 49 patients as part of conditioning regimens for bone marrow transplantation (3,4). Twenty-eight patients had no neurologic sequelae and 15 additional patients experienced only minimal neurologic symptoms (eg, transient lethargy, headache, possible hallucinations, and slurring of speech) which were not considered to be of clinical significance. One patient had symptoms related to a CNS hemorrhage and one patient who received 15 mg/kg of procarbazine every other day × 3 had persistent lethargy lasting 7 days, poor coordination, an abnormal mental status, and a diffusely abnormal electroencephalogram similar to the two patients described previously. Other investigators have also noted diffuse, nonspecific slow waves in electroencephalograms of patients receiving procarbazine (6).

Four patients received high-dose mechlorethamine therapy in addition to procarbazine before marrow transplantation. Two patients received mechlorethamine and procarbazine on different days and had no neurologic complications. The two patients described above both developed severe lethargy and disorientation lasting approximately 1 week after receiving high-dose procarbazine and mechlorethamine on the same day. The similarity of their clinical picture and the rarity of similar symptomatology in patients receiving procarbazine alone suggest that mechlorethamine may have potentiated the CNS depressant effects of procarbazine. Synergy of the neurotoxic manifestations of procarbazine has previously been noted with phenothiazines, barbiturates, narcotics, and tricyclic antidepressants (1,7).

At standard doses, serious neurologic toxicity after mechlorethamine administration is rare (2). Diffuse, slow waves on the electroencephalogram have been reported after intracarotid mechlorethamine administration (8). One recent case report (9) noted fever, coma, and seizures in one patient each of two times that he received 0.4 mg/kg of mechlorethamine.

The observation that both of our patients who received high doses of procarbazine and mechlorethamine on the same day developed marked somnolence suggests a possible drug interaction. Even though this cannot be proven, it would seem prudent to avoid administration of high-dose mechlorethamine and procarbazine on the same day, either in conditioning for marrow transplantation or in other high-dose chemotherapy regimens. The possible synergistic action of these drugs might be exploitable in future studies and should be considered as a cause of CNS disturbances in patients receiving these two drugs in high doses.

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Iv Methyl-CCNU, VM-26, and Cranial Irradiation in Therapy for Malignant Brain Tumors [1]

Brief Reports and Preliminary Communications

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The success of chemotherapeutic agents in combination against lymphoproliferative malignancies (1,2) has prompted the study of drug combinations for the treatment of brain tumors (3,4). Iv methyl-CCNU and VM-26 have demonstrated individual activity against malignant cerebral neoplasms, with each agent inducing acceptable systemic toxicity (5,6). This paper reports the results of a phase II trial of iv methyl-CCNU and VM-26 in combination against primary CNS glial tumors.

MATERIALS AND METHODS

Patient Selection

Twenty-two consecutive patients were admitted to the BCRC and entered into the study. To qualify for treatment a patient had to have a biopsy-proven cerebral glial neoplasm. When a biopsy was not possible, unequivocal radiographic and clinical findings of a glial tumor were acceptable.

Drug Administration and Supportive Measures

Methyl-CCNU was prepared in a fat-emulsion vehicle by a previously described technique (7). This methyl-CCNU suspension was administered iv via a Harvard infusion pump at the rate of 3 ml/minute. VM-26 was diluted with 150 ml of 5% dextrose in water and administered iv over 30 minutes. Chemotherapy was given in 6-week courses. On the first day of each course of chemotherapy the patient received iv methyl-CCNU (130–150 mg/m^2) followed by VM-26 (100 mg/m^2); thereafter VM-26 doses were administered weekly for the next 5 weeks. There was a 1-week rest period between the last VM-26 dose and the beginning of the next course of methyl-CCNU and VM-26.

Because corticosteroids may have an effect on survival, doses were strictly regulated, and frequent attempts were made to reduce the dose. Neurologic improvement achieved with a steroid dose increase was attributed to a reduction in cerebral edema, and, therefore, changes in the steroid dose were carefully noted when evaluating response to chemotherapy. Recognizing some experimental evidence to the contrary (8), we assumed that corticosteroids had no antitumor effect.

All patients accepted for study received cranial irradiation if none had been administered in the past. Megavoltage irradiation (5000–6000 rads) was given over the 6-week period concomitant with the first course of chemotherapy.

Patient Evaluation

Prior to each course of therapy patients were carefully evaluated by a detailed neurologic examination and their disease was staged from 1 (no deficit) to 4 (severe deficit) according to the CNS disease-staging system developed by Rosenblum et al which is based on a composite picture of function, symptoms, and signs (9). Irreversible, stable deficits secondary to surgery were not used in determining stage.

Response to chemotherapy was determined by comparing the stage prior to treatment with that just prior to the second course. Patients who were stage 1 (no deficit) prior to treatment and remained so at the end of one course of therapy were said to have "continued well being." If a patient improved in disease stage, he was considered to have a "response." Stability in a measurable deficit from course to course was designated "no change," and deterioration was called "progression." Survival and the interval between the beginning of chemotherapy and frank tumor re-growth (progression-free interval) were also studied in these patients.

RESULTS

Patient Population

Results were tabulated as of April 15, 1976. Twenty-two patients received at least one course of

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chemotherapy and were considered evaluable. Seven patients had glioblastomas and nine had malignant astrocytomas. Four patients had cerebral astrocytomas (grade 2, Kernohan), two of whom had been previously irradiated. One patient had a primary CNS lymphoma; his condition worsened quickly and he never received cranial irradiation. Another patient had had prior irradiation but no biopsy. She showed clinical and radiographic signs of a vascular left thalamic tumor which at autopsy was shown to be a ganglioglioma. This was the only patient who did not have at least a partial tumor resection before referral for this study. No patient had received prior chemotherapy.

Initial neurologic staging revealed varying degrees of impairment. Nine patients were grade 1, five were grade 2, and eight were grade 3. The 22 patients received an average of 3.5 doses of methyl-CCNU and 16.6 doses of VM-26. Six patients required methyl-CCNU dose reductions because of myelotoxicity.

Response to Therapy

Of the 13 patients who had clinically measurable disease (stages 2 and 3), three achieved objective remissions. These responses came in patients with the more aggressive tumors, malignant astrocytomas and glioblastomas. All improvement seen in these patients was noted during the first course of therapy, and this improvement persisted for 9, 17, and 56 weeks, respectively (progression-free interval).

All nine of the stage 1 patients had continued well being through the first course of therapy. Three patients were admitted with progressing disease and stabilized (no change). Seven of the 13 patients with measurable disease continued to deteriorate through the first and subsequent courses of therapy.

Six patients had more benign pathology and were eliminated from the consideration of patient survival. The other 16 patients were relatively homogeneous. All had malignant astrocytomas or glioblastomas and were referred for therapy between 2 and 16 weeks (median, 5.5 weeks and mean, 6 weeks) after a partial tumor resection. All patients had cranial irradiation (5000–6000 rads) concomitant with their first course of chemotherapy. In this group with malignant tumors, the median survival of all patients who may have had some response to therapy (response, continued well being, and no change) was 57 weeks (mean, 59 weeks), while those patients who clearly had no response (progression) had a median survival of 15 weeks (mean, 15 weeks). The median survival of all 16 patients with malignant astrocytomas or glioblastomas was 48 weeks (mean, 48 weeks).

Toxicity

Twelve patients (54%) demonstrated leukopenia (< 2000 cells/mm^3) after chemotherapy, with the wbc count nadir occurring at 3–8 weeks (mean, 4 weeks) after the start of a course of treatment. Eight of these patients developed leukopenia during their first course of treatment while the other patients had leukopenia during the second course which tended to persist through subsequent courses despite dose reductions. Thrombocytopenia (< 50,000 cells/mm^3) was seen in eight of the 12 patients who developed leukopenia. The platelet count nadirs occurred at 3–4 weeks (mean, 4 weeks) after the start of a treatment course. No serious infections or bleeding diatheses resulted from this hematologic toxicity, but one patient received prophylactic platelet transfusions.

DISCUSSION

Successful combination chemotherapy seems to depend upon the use of individually active agents with toxicity which allows them to be combined at relatively high doses (1,2). Methyl-CCNU and VM-26 showed moderate individual efficacy against malignant brain tumors (5,6), and the relative marrow-sparing properties of VM-26 made it a logical choice for combination with methyl-CCNU, a nitrosourea with typically potent delayed bone marrow toxicity. The toxicity of this combination, however, was somewhat more severe than could be predicted from the individual records of these agents. More than half of the patients treated with this regimen experienced severe myelosuppression.

Three of the 13 patients who had measurable disease improved while receiving combination therapy while three others stabilized from what was rapidly progressing disease. The overall response rate (48%) compares favorably to that seen with other drug combinations tested against brain tumors (3,4). However, the real strength of a study which dictates the delivery of antitumor therapy as a postoperative adjuvant lies in its ability to gather survival data. Studying a large group of patients selected by criteria similar to ours, the Brain Tumor Study Group has established that patients with malignant brain tumors treated with surgery, irradiation, and BCNU have a median survival of 51 weeks (10). The 16 patients in our study who had definitive resection of a malignant tumor followed by radiation, methyl-CCNU, and VM-26 had a median survival of 48 weeks. This suggests that this

combination, like others tested against brain tumors, has antitumor activity that offers no advantage over single-agent therapy with BCNU (4).

Combination chemotherapy has not yet offered a dramatic advance in the treatment of malignant brain tumors. This may be because the individual agents used in the various combinations have only marginal antitumor activity in brain tumors. New agents with the necessary properties to be active in the CNS are needed (11). More detailed knowledge of the pharmacology of antitumor agents and the biochemistry and cell kinetics of their target tumors may suggest new approaches to the design of drug combinations (1,2). As expertise in these areas grows, the combination chemotherapy for malignant brain tumors will undoubtedly improve.

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Phase II Study of Azotomycin in Sarcomas [1]

Paul Chang* and Peter H. Wiernik [2]

Brief Reports and Preliminary Communications

Azotomycin is an antitumor antibiotic which was isolated from the bacteria *Streptomyces ambofaciens*. The drug is one of a group of diazo analogs of L-glutamine, all of which are thought to exert an antitumor effect by interfering with steps which require glutamine in the biosynthesis of purines (1).

In a broad phase II study, azotomycin therapy resulted in > 50% tumor regression in four of 16 evaluable patients with soft tissue sarcomas (2). Subsequently, a technical problem in the manufacture of the drug forced its withdrawal from further testing.[3] Because of the apparent activity identified in the initial trials, it was decided to attempt a tumor-specific phase II trial in patients with sarcomas to determine the desirability of obtaining a new source for this chemical.

MATERIALS AND METHODS

Thirteen patients with histologically documented inoperable or metastatic sarcomas received 80–120 mg/m^2 of azotomycin by rapid iv injection daily for 10 days or less if intolerable toxicity intervened. All patients had measurable lesions, and had failed prior therapy with adriamycin. Most patients had also received other drugs, and three patients had had extensive prior radiation therapy (table 1). All patients had wbc counts > 4000/μl, platelet counts > 100,000/μl, and normal serum BUN or creatinine concentrations prior to initiation of therapy. All but three of the patients were ambulatory prior to treatment. Courses were repeated every 3 weeks if drug toxicity had subsided.

Partial response was defined as a > 50% decrease in the sum of the products of the perpendicular diameters of all measurable lesions for at least 4 weeks. Any objective increase in this sum after two courses was defined as progressive disease. No change in tumor size after two courses was considered to be no response since some of these patients had very slowly progressive disease.

RESULTS

Only one patient, a 66-year-old white man with an extraskeletal pelvic myxoid chondrosarcoma, had a slight decrease in the size of his pelvic mass after one course and had achieved a partial response with additional tumor shrinkage and loss of peripheral edema after two courses. Nausea and vomiting were so poorly controllable, however, that the patient refused to undergo further treatment and he died 3 months later.

The major toxic effect of this drug was severe nausea and vomiting in all patients. This resulted in termination of treatment before the full 10-day course was administered in 17 of the 23 courses given. Six of the 13 patients required hospitalization for re-hydration despite antiemetics, careful monitoring of fluid status, and daily parenteral fluid supplementation. In addition, the transient azotemia seen in ten patients was felt to be pre-renal in origin. With re-hydration, renal function returned to normal in all ten patients.

Myelosuppression was usually only moderate. The median wbc count nadir was 2600/μl (range, 600–6600/μl) occurring on Day 10 with recovery (wbcs > 2000/μl) in 2–3 days. The median platelet count nadir of 122,000/μl (range, 16,000–330,000/μl) was reached on Day 12 with recovery (platelets > 100,000/μl) usually within 3 days. Three patients died of drug-related side effects, two of sepsis superimposed on prolonged periods of granulocytopenia and one of probable hypovolemic shock with aspiration pneumonia after severe vomiting.

COMMENTARY

Azotomycin appears to have very little therapeutic activity against human sarcomas. With the same

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TABLE 1.—Patient characteristics and clinical results of azotomycin therapy

Patient No.	Age/sex/race*	Diagnosis (primary site)	Metastases	PS†	Prior therapy‡	Dose (mg/m²/day) × No. of days given	Response
1	55/F/W	Giant cell tumor (ribs)	Lungs	85	A, M, S, C, V, B	100 × 6, 100 × 5	Progression
2	65/M/W	Leiomyosarcoma (abdominal wall)	Lungs	50	RT, A, S, C, V, D	80 × 6, 80 × 6, 80 × 5	Progression
3	18/M/B	Synovial sarcoma (leg)	Lungs	60	A	100 × 7, 120 × 6	Progression
4	28/M/W	Synovial sarcoma (leg)	Lungs	90	A, C	120 × 6, 120 × 6, 100 × 10	Progression
5	66/M/W	Chondrosarcoma (pelvis)	Lungs, thigh	30	A, M	120 × 6, 100 × 8	Partial
6	38/M/W	Alveolar soft part sarcoma (arm)	Lungs, scapula	70	A, P	100 × 6, 100 × 10	None
7	57/F/W	Malignant fibrous histiocytoma (spine)	Subcutaneous	30	A, C, V, B	100 × 7	None (died on Day 9—infected)
8	61/M/W	Leiomyosarcoma (retroperitoneum)	None	50	A, C, V, D	100 × 10	Progression
9	54/F/W	Leiomyosarcoma (terminal ileum)	Liver	80	A, C, V, D	100 × 10, 100 × 8	Progression
10	62/F/W	Liposarcoma (retroperitoneum)	None	50	A, S	100 × 6	Progression (died on Day 7—shock)
11	55/F/W	Leiomyosarcoma (uterus)	Lungs	30	A, S, RT	100 × 8	None (died on Day 20—infected)
12	66/M/B	Leiomyosarcoma (stomach)	Liver	70	A, C, V, D	100 × 4	Progression
13	52/F/B	Leiomyosarcoma (retroperitoneum)	None	70	A, C, V, D, RT	100 × 10, 100 × 10	Progression

*W = white; B = black.

†Karnofsky performance status.

‡A = adriamycin, M = methyl-CCNU, S = streptozotocin, C = cyclophosphamide, B = bleomycin, V = vincristine, D = DTIC, P = poly I:C lysine, and RT = radiation therapy.

dose and schedule of azotomycin that were used in the original phase II study of this drug, we noted rather marked, therapy-limiting nausea and vomiting, but with only minimal tumor response. Further chemotherapeutic trials with this drug do not appear warranted.

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Treatment of Refractory Adult Acute Nonlymphoblastic Leukemia With Subcutaneous 5-Azacytidine[1,2]

Brief Reports and Preliminary Communications

James O. Armitage and C. Patrick Burns[3,4,*]

The pyrimidine analog 5-azacytidine has been known for some time to be active in adult acute nonlymphoblastic leukemia (ANLL). However, the use of the drug has been limited at least partly because of its toxicity. Severe gastrointestinal toxicity manifested as nausea, vomiting, and diarrhea has been a limiting factor in some studies when 5-azacytidine was given on an intermittent, iv schedule (1). In contrast, only mild gastrointestinal toxicity was noted during a trial of sc administered 5-azacytidine in patients with advanced solid tumors (2). In addition, sc administration of 5-azacytidine gives blood levels comparable to iv administration, albeit delayed by approximately 2 hours (3). Therefore, we undertook a pilot study of sc administered 5-azacytidine in adults with ANLL refractory to other therapies.

PATIENTS AND METHODS

The clinical characteristics, subtype of ANLL, and previous induction therapies of the ten patients in this study are listed in table 1. The patients' ages ranged from 19 to 66 years. Seven of the patients had previously been in complete remission (CR) with a median first remission duration of 14 months. All patients were refractory to cytosine arabinoside and daunorubicin before they received 5-azacytidine. Patients were required to have a creatinine level of < 2.0 mg/100 ml, a bilirubin level of < 1.5 mg/100 ml, and no evidence of mass lesions in the liver for entry into the study. No patients were infected at the time of the first dose of 5-azacytidine.

5-Azacytidine was supplied by the Division of Cancer Treatment of the National Cancer Institute (Bethesda, Md). The drug was administered sc at a dosage of 100 mg/m^2 every 12 hours for 5 days. For administration, 5-azacytidine was dissolved in 3 ml of sterile water or lactated Ringer's solution per 100 mg of drug and injected sc into the anterior thigh within 15 minutes of reconstitution. Since all patients were > 1 m^2 and the maximum volume injected at one site was 3 ml, it was necessary to use two injections per dose. A bone marrow was obtained 9 days after each course, and the sequence was repeated if no antileukemic effect was evident. Patients who had no response after two courses were removed from the study. Patients having a partial remission (PR) after two courses were given a third course in an attempt to induce CR. All patients received vigorous supportive care including random donor or HLA-matched platelets given for acute hemorrhage or prophylactically if the platelet count was $< 20{,}000$/mm^3, and granulocyte transfusions when appropriate. The criteria for response were those of Acute Leukemia Group B.[5] All patients were considered evaluable. Survival was calculated as the time from the first administration of 5-azacytidine to death.

RESULTS

The number of courses of 5-azacytidine administered, the result, and the survival for these ten patients are presented in table 1. Two patients achieved the criteria for a PR and survived for 409 and 128 days. The median survival for the patients not achieving PR was 85 days. Leukemic blast cells were cleared from the peripheral blood in seven patients and were strikingly reduced in the bone marrow in three patients. One of the latter patients died from infection during the aplasia following therapy and had no residual leukemia at autopsy.

Hematologic toxicity was severe with all patients requiring rbc and platelet transfusions and four patients received transfused granulocytes. The me-

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[4]We gratefully acknowledge the assistance of house officers, fellows, and attending staff of the Department of Medicine who participated in the care of our patients.

[*]*Reprint requests to:* Dr. C. Patrick Burns, Department of Medicine, University Hospitals, Iowa City, Iowa 52242.

[5]Acute Leukemia Group B (ALGB). Criteria for evaluating acute leukemia of the Acute Leukemia Cooperative Group B (June 1974 modification of 1969 criteria). Scarsdale, NY, ALGB Operations Office.

TABLE 1.—Characteristics of patients treated with sc 5-azacytidine

Patient No.	Age/sex	Diagnosis*	Previous treatment†	Result‡	Duration (mos)	No. of courses of 5-azacytidine	Result‡	Survival (days)
1	28/M	AML	AT	CR	20			
			TADPO	CR	5	3	PR	128
			TADPO	NR	—			
2	66/F	AML	TADPO	NR	—	3	PR	409
3	35/M	AML	AT	CR	38			
			D	CR	3	2	NR	141
			CAM	NR	—			
4	19/F	AMML	AT	CR	15	2	NR	55
			D	NR	—			
5	24/M	AEL	DP	CR	14	1	NR	292+
			D	NR	—			
6	26/M	AML	AT	CR	8	2	NR	69
			TAD	NR	—			
7	37/M	AML	TADPO	NR	—	2	NR	102
			AT	CR	3			
8	50/F	AMML	TADPO	CR	3	1	NR	8
			TADPO	NR	—			
9	46/F	AML	AT	NR	—	2	NR	47
			D	NR	—			
10	26/F	AML	AT	NR	—	1	NR	156
			D	NR	—			

*AML = acute myeloblastic leukemia; AMML = acute myelomonocytic leukemia; and AEL = acute erythroleukemia.
†A = cytosine arabinoside; T = 6-thioguanine; D = daunorubicin; P = prednisone; O= vincristine; C = cyclophosphamide; and M = methotrexate.
‡NR = no remission.

dian platelet and neutrophil nadir counts were 6000/mm^3 and 0/mm^3, respectively. The time to nadir (from first day of treatment) varied from 8 to 20 days, and the duration of nadir varied from 21 to 35 days.

Gastrointestinal toxicity manifested as mild-to-moderate nausea and vomiting during the first 1–3 days of each treatment occurred in all patients. This symptom began within 1–2 hours and often had not subsided prior to the next dose. The nausea and vomiting diminished with repeat doses in all but two patients. Although the nausea and vomiting was generally easily controlled with parenteral antiemetics and did not limit the use of the drug, one patient was felt to require iv fluid replacement. Hepatic toxicity manifested as elevated bilirubin (peak level ranging from 1.6 to 2.5 mg/100 ml) and SGOT levels was noted in three patients. These toxic effects resolved promptly at the end of each course in which they occurred. No patient developed stomatitis and no neurotoxicity was observed.

Patients reported a moderately severe burning pain with injections and a slightly raised, violaceous, tender skin lesion occurred at the injection sites in all but two patients. These skin lesions appeared within 1–3 hours and subsided in 5–10 days. Biopsy of the lesions revealed a perivascular lymphohistiocytic infiltrate. There were a few neutrophils but no eosinophils seen. There was no permanent induration or scarring at the sites; however, most patients had a persistent brown skin discoloration in the area of the rash.

DISCUSSION

The antileukemic activity and hematologic toxicity noted in this study indicate that active drug is absorbed after sc administration of 5-azacytidine. However, the rate of response of refractory ANLL is lower than other investigators have seen after iv administration of the drug. The pooled results of 200 patients with ANLL treated with 5-azacytidine revealed 20% CR and 16% PR rates (4). Within a wide range of dosages there was no apparent dose-response curve. Vogler et al, using 5-azacytidine by continuous iv infusion in doses ranging from 100 to 300 mg/m^2/day, found 24% CR and 9% PR rates in 45 patients (5). Although the number of patients treated in our study is too small to draw definite conclusions, it seems unlikely that the response

rate with sc administration is higher than that for iv administration.

This study confirms the previous observations of milder nausea and vomiting that accompanies the sc administration of 5-azacytidine (2). While there is less gastrointestinal toxicity when the drug is given in this manner than has been reported for intermittent iv administration (1), it is now known that iv administration by continuous infusion can also decrease nausea and vomiting (5).

The potential uses for the sc route in administering 5-azacytidine appear limited. From the results reported to date it would appear that continuous iv infusion would be the preferred route administration in most instances. However, for patients in whom iv administration of drugs is difficult or impossible, and for maintenance therapy where continuous iv administration would require otherwise unnecessary hospitalization, the sc route may be a useful alternative.

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Phase II Study of Cytosine Arabinoside in Advanced Large Bowel Cancer [1,2]

Brief Reports and Preliminary Communications

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Cytosine arabinoside (ara-C) has been demonstrated to be an effective agent in the treatment of human acute myeloblastic leukemia (1). Studies from the Southern Research Institute on certain transplanted colonic adenocarcinomas in mice have shown ara-C to have significant activity in this model system (2). Early trials have suggested potential activity of this drug in human large bowel carcinoma (3,4). The present study was undertaken to further define the activity of this compound in human colorectal cancer.

MATERIALS AND METHODS

Patients.—Thirty-two patients with measurable metastatic colorectal carcinoma received treatment. All were acceptable candidates for cytotoxic therapy with respect to hematologic status (wbc count $> 4000/mm^3$, platelet count $> 130{,}000/mm^3$). No patient had received any therapy for at least 30 days prior to receiving ara-C. All patients were ambulatory although 11 of 32 patients treated had significant impairment of their activity level (Eastern Cooperative Oncology Group [ECOG] performance score 2 or 3). Sixteen patients had received prior chemotherapy. Of these, two had also received prior radiation therapy, and nine had received prior immunotherapy.

Drug administration.—Ara-C was administered by rapid iv injection twice daily × 5 days at an initial dose of 100 mg/m^2 for each injection.

Evaluation.—Prior to each treatment, patients had a general examination, chest roentgenogram, hemogram, and liver function studies performed. The following immunologic parameters were also secured: recall and dinitrochlorobenzene skin tests; lymphocyte blastogenesis with phytohemagglutinin, concanavalin A, and pokeweed mitogen; immunoglobulins IgA, IgM, and IgG; and circulating lymphocytes, T cells, and B cells as previously described (5). After treatment, leukocyte counts were obtained twice weekly and platelet counts once weekly for the first 4 weeks and then weekly thereafter. Patients who demonstrated objective response or stability were re-treated at 4 weeks, and then at regular 5-week intervals until progression.

Objective response was defined as a decrease of at least 50% in the product of the perpendicular diameters of the most clearly measurable indicator lesion for a minimum of 4 weeks. If hepatomegaly was the primary indicator, there had to be a reduction of the sum of liver measurements below each costal margin at the midclavicular line and xiphoid process by at least 30%. In addition, there could be no new areas of malignant disease or significant deterioration in weight (> 10%) or performance status (more than one score level, ECOG). Progression of disease was defined as an increase in measurable lesions by > 25%, appearance of new areas of malignant disease, decrease in weight > 10%, or decrease in performance status more than one level (ECOG).

THERAPEUTIC RESULTS

Responses.—No patient achieved an objective response. At 8 weeks after initiation of treatment, 22 patients progressed and ten patients remained stable for a median duration of 95 days. Median survival for the overall patient population was 116 days (range, 10–279+ days).

Toxicity.—Toxic effects from ara-C were primarily hematologic and gastrointestinal (table 1). Nausea occurred in 25 of 32 patients with concurrent vomiting in 21 patients (severe in five patients). Diarrhea occurred in 13 patients, being severe in three. Stomatitis of a mild degree was noted in eight patients. Leukopenia (< 4000 cells/mm^3) was experienced by 22 of the 32 patients (69%) and thrombocytopenia ($< 130{,}000$ cells/mm^3) was experienced by 12 patients (40%).

Immunologic parameters.—Patients were ana-

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TABLE 1.—Toxic reactions in 32 patients

Toxic reaction	Median (range)
Hematologic*	
Leukopenia	
Nadir (cells/mm³)	2500 (600–9200)
Day of nadir† (wbcs < 4000)	20 (15–25)
Day of recovery	26 (20–30)
Thrombocytopenia	
Nadir (cells/mm³)	151,000 (10,000–463,000)
Day of nadir (platelets < 130,000)	12 (10–18)
Day of recovery	17 (15–27)
Other	*No. of patients*
Nausea	25
Vomiting	21
Diarrhea	13
Stomatitis	8

*Tabulated for 1st cycle of therapy.
†Day of nadir and recovery calculated from 1st day of treatment.

lyzed in two groups as determined by objective status at 8 weeks, ie, objectively stable or progressed. The pretreatment immunologic determinants were of no apparent prognostic value in discriminating between patient categories.

DISCUSSION

Ara-C has been shown to have a high degree of antitumor activity against 1,2-dimethylhydrazine-induced transplantable murine colon adenocarcinoma in BALB/c mice and a lower degree of activity against a similar tumor induced in C57BL/6 mice (2). However, rapid tumor proliferation observed in these model systems suggests kinetic differences from human colorectal carcinomas which may account for the efficacy of the cycle-dependent agent ara-C in the animal experimental setting and the lack of therapeutic effect in the clinical setting.

In an earlier phase II study, Davis and associates (4) found a partial response in 11 of 97 patients (12%) with colorectal cancer treated with ara-C given by rapid injection in dosage schedules ranging from 0.8 to 3.5 mg/kg/day × 10 days. None of the responses were of a long duration nor did they result in any substantial clinical benefit.

In our study utilizing a dose of 100 mg/m² twice daily × 5 days we did not observe any objective responses among 32 patients. Ara-C given in this moderately toxic dosage schedule is not effective in human colorectal carcinoma.

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Phase II Study of Vindesine Sulfate in the Therapy for Advanced Renal Carcinoma [1,2,3]

Brief Reports and Preliminary Communications

Peter P. Wong, Alan Yagoda,* Violante E. Currie, and Charles W. Young [4,5]

Vindesine sulfate is a new agent derived from the vinca alkaloid, vinblastine (VLB). Vindesine was found in animal studies to possess antitumor activity similar to that of vincristine (VCR) but exhibited no neurotoxicity.[6] Extensive phase I trials by five institutions are evaluating various dosage schedules.[7] Initial clinical trials at Memorial Sloan-Kettering Cancer Center used two iv schedules: weekly and for 5 consecutive days every 3–4 weeks.[8]

Although renal cell carcinoma is relatively refractory to most chemotherapeutic agents, the vinca alkaloids, VCR and VLB, have been shown to induce objective remissions in 5%–15% of patients with this tumor (1,2). Therefore, a phase II disease-oriented study was undertaken in patients with advanced hypernephroma and forms the basis of this report.

MATERIALS AND METHODS

Twenty-two patients with measurable, unresectable hypernephroma received vindesine. Pathologic material from 20 patients was reviewed by our Department of Pathology. The diagnosis of renal cell cancer was accepted in two patients who had selective renal angiography, aortography, and inferior and/or superior venography which delineated hypervascular masses characteristic of hypernephroma in the upper pole of the right kidney. Both patients had larger masses, 10.5 × 11.5 cm and 15 × 10 cm, with neovascularity and evidence of measurable metastatic disease in other sites. Patients had a complete history and physical examination with measurement of all objective lesions by two diameters. Laboratory evaluation included cbc counts which were obtained weekly or daily depending on the drug schedule used, a 12-channel screening profile, 5′-nucleotidase, and chest and skeletal roentgenograms. In selected patients, liver, bone, and brain radioisotopic scans were obtained at designated intervals to evaluate response. Toxicity was evaluated in most patients weekly while response was evaluated at 2–4-week intervals. Response criteria included partial remission (PR) denoting a > 50% reduction in the sum of the product of the perpendicular diameters of all measurable lesions for > 1 month; minor remission signifying a > 25% decrease in measurable lesions or PR status of < 1-month duration; and stabilization meaning a ≤ 25% change in tumor size. The Karnofsky performance status (PS) was used to evaluate subjective changes.

An adequate trial was defined as 1-month survival with hematologic toxicity and evidence of progression of disease after a total dose of > 8 mg/m² in patients receiving the weekly dose or two courses in patients receiving the 5-day schedule.

Vindesine was supplied in sterile vials containing 10 mg of lyophilized powder, and was dissolved in a diluent containing sodium chloride, benzyl alcohol, and water to a concentration of 1 mg/ml before administration. The two iv schedules used for vindesine were 3–4 mg/m² weekly (19 patients) and 1.0–1.3 mg/m² daily for 5 days (three patients). The initial dose depended upon the patients' PS, hepatic functions, extent of prior irradiation, and sensitivity to other myelosuppressive agents. The initial dose of vindesine was decreased by 50% if the PS was < 50, the serum bilirubin was > 2.0 mg/100 ml, or

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[3]Vindesine sulfate: NSC-245467; CAS reg. No. 59917-39-4; vincaleukoblastine, 3-(aminocarbonyl)-*O*⁴-deacetyl-3-de(methoxycarbonyl)-, sulfate (1:1) (salt), mixt. with D-mannitol. Supplied by Eli Lilly and Co, Indianapolis, Ind.

[4]Solid Tumor Service (Drs. Wong and Yagoda) and Clinical Chemotherapy Service (Drs. Currie and Young), Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.

[5]We gratefully acknowledge the technical assistance of Mrs. Laura Lapp and the secretarial assistance of Mrs. Isa Irvin in the preparation of this manuscript.

[6]Compound 99094, desacetyl vinblastine amide sulfate, an investigational new drug. Information for clinical investigators. Prepared by Eli Lilly and Co, Indianapolis, Ind, Apr 14, 1975.

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the patient had received > 4000 rads of radiation to the pelvis or spine. Subsequently, doses were modified upward or downward depending upon toxicity and/or response.

Patient Characteristics

The pathology was clear cell hypernephroma in 16 patients, mixed clear cell and granular in two patients, and adenocarcinoma in two patients. The median age was 58 years (range, 38–73 years) and the median PS was 60 (range, 40–100). There were 11 women and 11 men. The median time from symptoms to diagnosis, diagnosis to metastasis, and diagnosis to protocol was 8 (range, 0–106), 24 (range, 0–260), and 40 (range, 0–300) weeks, respectively. Seventeen (74%) patients had had prior radiation, five had had postoperative radiation, and 12 had received radiation to the metastatic sites. The median time from radiation therapy to protocol was 1 week (range, 0–48 weeks). Seven (31%) patients had received progestins, three (14%) immunotherapy, and five (22%) chemotherapy, of which two had had VCR. Thus, 78% of patients had had no prior chemotherapy. Sites of evaluable lesions included pulmonary (90%), hepatic (50%), bone (27%), brain (14%), abdomen (14%), and nodes or subcutaneous masses (14%). Five (22%) patients had hypercalcemia and four of these showed no demonstrable bony lesions by radioisotopic scans or skeletal surveys.

RESULTS

Five patients did not have an adequate trial; three patients died within 1 month, one patient refused further vindesine after two doses because of nausea and vomiting, and one patient was lost to followup after a single dose. The median PS in this group was 50 and two had had prior chemotherapy.

Of the 17 patients who were evaluable, 14 received weekly vindesine for an average of 6 weeks (median, 5 weeks; range, 2–12 weeks). The average total dose was 19 mg/m^2 (median, 17 mg/m^2; range, 8.0–40 mg/m^2). Each of the three patients who received five consecutive days of vindesine had two courses with an average of 5.3 mg/m^2/course.

Toxicity

Nineteen patients were evaluable for toxicity (table 1). A majority of patients receiving the weekly schedule complained of fever associated with malaise and myalgias which began 2–4 hours after each injection and lasted up to 24 hours. Occasionally, the subjective symptom of lethargy would continue for several days. Phenacetin promptly lowered the vindesine-induced fever. Some degree of hair loss was almost universal and in 70% of patients, partial or complete alopecia occurred. Constipation and obstipation heralding paralytic ileus occurred in two patients; most of the group had received prophylactic measures such as stool softeners. Neurotoxicity was prominent and resembled that associated with VCR. One patient had proximal muscle weakness, particularly of the lower extremities, after a total dose of 15.5 mg/m^2. He noted difficulty in arising from a sitting position which resolved spontaneously 6 weeks later. A needle electromyelogram indicated a mild lower motor neuron dysfunction in this patient.

TABLE 1.—Toxicity of vindesine (19 patients)

Toxic effect	No. of patients (%)
Leukopenia (wbcs/mm^3)	
< 4000 > 2000	10 (53)
< 2000	6 (32)
Thrombocytopenia (platelets/mm^3)	
< 200,000 > 100,000	6 (32)
< 100,000	1 (5)
Neurotoxicity	
Paresthesias	13 (70)
Peripheral neuropathy	9 (47)
Constipation	2 (10)
Jaw pain	2 (10)
Miscellaneous	
Alopecia	13 (70)
Fever	12 (62)
Malaise/myalgias	9 (47)
Nausea	2 (10)

Sixteen patients (85%) had leukopenia with a median nadir of 2.2 cells × 10^3/mm^3 (range, 0.5–3.4 cells × 10^3/mm^3). Leukopenia generally occurred 7–12 days after the second or third weekly dose with recovery within the following week, while wbc count nadirs with the 5-day course were noted 10 days later, with normalization within 3 weeks. One patient who received two weekly doses (total dose, 8 mg/m^2) had severe leukopenia of 500 cells/mm^3 and succumbed to sepsis and hepatic failure despite appropriate therapy and return of her wbc count to 5000/mm^3. Her only abnormal liver function test was a 5′-nucleotidase of 72 units (normal, 3–14 units), which suggested an early component of biliary obstruction. This was the only drug-related death.

Thrombocytopenia was seen in seven (37%) patients (table 1) and was never dose-limiting.

TABLE 2.—Therapeutic effect of vindesine in advanced hypernephroma

No. of patients entered	No. of patients with adequate trial	No. of responses (mos)		
		PR	Minor	Stabilization
22	17	0	2(1½, 1)	4(8, 5½, 2½, 2)

Therapeutic Effect

Two of 17 patients (12%) obtained minor response status; one patient had regression of pulmonary and hepatic metastases for 6 weeks and in one patient pulmonary nodules regressed for 1 month (table 2). Four additional patients showed stabilization for 2, 2½, 5½, and 8 months. Seventeen of 22 patients have died with a median duration from protocol to last followup of 2½ months (range, < 1–10+ months).

DISCUSSION

Vindesine does not appear to be an active agent in the treatment of advanced renal cell carcinoma. This drug can produce significant myelosuppression similar to VLB and moderate neurotoxicity and platelet sparing similar to VCR. Since other vinca alkaloids have some biliary excretion (3), dosage modification may be necessary with vindesine in patients having evidence of obstructive hepatopathy.

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Piperazinedione in Metastatic Renal Carcinoma [1,2]

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For the New Drug Committee, Cancer and Leukemia Group B

Piperazinedione, an agent which inhibits DNA synthesis, has been shown in phase I studies in man to have some antitumor effect in melanoma, renal cell carcinoma, and prostatic carcinoma[4] with acceptable toxicity including myelosuppression, nausea, vomiting, and fever. Since disseminated renal cell carcinoma had proven essentially refractory to chemotherapy (1), the Cancer and Leukemia Group B undertook a test of the efficacy of piperazinedione in renal cell carcinoma.

MATERIALS AND METHODS

Patient selection.—Patients with biopsy-proven renal cell carcinoma and measurable evidence of metastatic disease were considered eligible for therapy regardless of former treatment or age. No chemotherapy or radiotherapy was permitted within 3 weeks prior to admission to study. Adequate bone marrow function was present as defined by a wbc count $> 4000/mm^3$ and a platelet count $> 100,000/mm^3$. Patients with serum bilirubin levels > 1.5 mg/100 ml and/or creatinine levels > 2.0 mg/100 ml were not admitted to the study.

Drug administration.—Piperazinedione was given in a dose of 12 mg/m^2 iv bolus every 28 days. Dose adjustments were made for hematologic toxicity. They are shown in table 1. Dose adjustments were made for peripheral blood cell counts both at the nadir and at the day of treatment.

Response evaluation.—Therapeutic responses were evaluated in the following manner: complete response was defined as total regression of all measurable lesions, partial response as a measurable decrease $> 50\%$, and improvement as any response less than a partial response.

RESULTS

Response

Twenty-three patients were treated according to this protocol. There were no complete or partial responses. Three patients had progressive disease following one course of therapy. The remaining patients demonstrated no improvement. Three patients were considered nonevaluable because they received only one dose of piperazinedione; two of these patients refused further medication for personal reasons unrelated to the side effects of medication and the other patient was unable to tolerate the medication due to gastrointestinal symptoms.

Of the 20 evaluable patients, 11 were previously untreated, four had received prior chemotherapy, three had had prior hormonal therapy, and two had received both hormones and chemotherapy.

Toxicity

Hematologic toxicity is shown in table 2. The toxicity consisted of leukopenia in nine patients and thrombocytopenia in nine patients; seven patients had both. In no patient did leukopenia precipitate infection. In two patients there was coincident bleeding and thrombocytopenia. In neither situation was the bleeding marked or life-threatening. Usually the blood cell counts returned to normal within 28 days; however, in several instances they required > 4 months.

There was minimal nonhematologic toxicity. Gastrointestinal toxicity was mild. Patients only occasionally complained of any nausea, vomiting, or diarrhea, and these complaints were usually mini-

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TABLE 1.—Dose adjustment

Wbc count (cells/mm³)		Platelet count (cells/mm³)	Dose adjustment
		At nadir	
> 3000	and	> 100,000	10% (increment)
< 1500	or	< 50,000	25% (reduction)
		At day of treatment	
> 4000		> 100,000	Full
3000–4000		75,000–100,000	75% (reduction)
2000–3000		50,000–75,000	50% (reduction)
< 2000		< 50,000	No treatment until recovery

TABLE 2.—Hematologic toxicity

	No. of patients
Wbc count (cells/mm³)	
< 3000 but > 2000	6
< 2000 but > 1000	3
< 1000	0
Platelet count (cells/mm³)	
100,000–50,000	3
50,000–25,000	4
< 24,000	2

mal. Hepatic toxicity consisted of two patients having minimal elevation of serum alkaline phosphatase and one patient having mild elevation of serum bilirubin. Two of these patients had metastatic disease to the liver and these changes may have been secondary to advancing disease rather than the effect of the drug. There was no oral ulceration. There was no evidence of renal, central nervous system, or respiratory toxicity.

DISCUSSION

This study presents no evidence that piperazinedione has an antitumor effect in advanced renal cancer. There were no objective responses in 20 evaluable patients.

The drug was reasonably well tolerated. There was little evidence of nonhematologic toxicity. Hematologic toxicity included leukopenia and/or thrombocytopenia. In several patients the duration of the myelosuppression was prolonged. Leukopenia was never associated with infection. In two patients, thrombocytopenia was associated with bleeding, but in neither situation was the bleeding marked or life-threatening.

Piperazinedione would appear to have no therapeutic use in renal cell carcinoma. It is being tested in other malignancies. It produces minimal nonhematologic toxicity and the hematologic toxicity is manageable.

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Phase II Trial of Pyrazofurin in Malignant Melanoma [1,2]

Daniel Budman, Violante Currie, and Robert Wittes [3,*]

Brief Reports and Preliminary Communications

Malignant melanoma is a disease whose incidence is increasing at a greater rate than any other neoplasm except lung cancer (1). Since melanoma is relatively resistant to available chemotherapeutic agents (2), there is a great need for therapeutic trials of new agents.

Pyrazofurin, a 3-β-D-ribofuranosyl-4-hydroxypyrazole-5-carboxamide, is a new antibiotic with both antiviral and cytolytic activity in animal solid tumor model systems. These effects may be the result of inhibition of de novo pyrimidine biosynthesis (3). In phase I trials, a once weekly dosage schedule produced dose-limiting mucositis and modest thrombocytopenia.[4] The present study was designed to evaluate the usefulness of this drug in malignant melanoma.

Twenty-one patients with histologically proven recurrent melanoma who had failed surgical attempts for cure were entered into the trial. Patients with an estimated survival time of < 1 month and/or hematologic compromise (wbc count, < 3000/mm³; platelet count, < 100,000/mm³) were excluded. All patients had easily measurable disease (table 1). The extent of disease was evaluated in each patient at the time of entry into the study by physical examination, chest roentgenogram, appropriate blood tests, and scintiscans when indicated.

An adequate trial of therapy was defined as a minimum of two doses of at least 4.0 mg/kg of body weight at weekly intervals. If no toxicity was observed, the dose was increased by 1.0 mg/kg at weekly intervals to a maximum of 7.0 mg/kg. The drug was given by rapid iv infusion after being dissolved in normal saline.

Categories of response have been previously defined (4). Patients were evaluated at weekly intervals for changes in measurable disease and performance status.

As indicated in table 1, 19 of the 21 patients were evaluable. The median performance status prior to therapy was reasonably high at 70. However, 90%

TABLE 1.—Characteristics and results of therapy in adequately treated patients

Characteristic	
Median age in yrs (range)	48 (33–68)
Sex	
Men	13
Women	6
Median initial performance status (range)	70 (30–90)
No. of patients	
Prior chemotherapy	
DTIC	16
Methyl-CCNU	1
None	2
Sites of known disease	
Bone	2
Central nervous system	4
Liver	7
Lung	16
Soft tissue/skin/node	16
Sites of measurable disease	
Liver	5
Lung	16
Soft tissue/skin/node	15
No. of courses of pyrazofurin given	
2	6
3	4
4	5
≥5	4
Toxic effect from treatment	
Mucositis	6
Leukopenia (≤ 4000 cells/mm³)	1
Thrombocytopenia	
≤ 50,000 cells/mm³	1
> 50,000 but ≤ 100,000 cells/mm³	3
Rash	1
Response to therapy	
Complete	0
Partial	0
Progression	19

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[4]Currie V, and Young CW. Manuscript in preparation.

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of patients had had prior exposure to either DTIC or a nitrosourea, and 18 of the 19 had significant visceral disease. Toxicity occurred in approximately one fourth of patients but was never life-threatening (table 1). No instances of either objective response or stabilization of disease were noted in any of the patients.

Based upon the present study, we believe that pyrazofurin has no role in the treatment of patients with visceral-dominant malignant melanoma who have received prior chemotherapy.

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LETTERS

Vinblastine-Related Pain in Tumors[1]

Vinblastine has been used in the treatment of a variety of neoplastic conditions (1). We have used vinblastine in combination with CCNU, bleomycin, and methotrexate for advanced squamous cell carcinoma of the head and neck. Thirty patients have been treated to date, 12 of whom (40%) have experienced severe tumor pain. In searching the literature for reports of vinblastine-related pain, we find a lack of thorough reporting except for a brief description by Stark and Fletcher (2).

CASE REPORTS

Patient 1

Patient 1 was a 44-year-old black man with recurrent squamous cell carcinoma of the floor of the mouth with extension to the left submental lymph nodes. His disease was originally managed with surgery followed by postoperative radiotherapy in September 1974. Disease recurred in December 1975, and was managed with combination chemotherapy consisting of vinblastine (15 mg iv), CCNU, methotrexate, and bleomycin. The patient received vinblastine by iv push before other agents were administered. Two to 3 minutes after injection the patient became restless, grabbed the tumor site by hand, and began to perspire profusely. He stated that he felt as if someone had poured "liquid fire" on his face and neck where the tumor was present. Fifty mg of im meperidine was given without relief. Repeated 5-mg doses of iv morphine sulfate slowly reduced the pain. Vital signs remained unchanged during this period. The patient returned to the clinic 1 month later for a second course of therapy. One-half hour before vinblastine injection, 10 mg of im morphine sulfate was given. An iv drip was started and 15 ml of normal saline was first infused which caused no pain. Fifteen minutes later, 15 mg of vinblastine solution in 15-ml volume was given by push. Three minutes after the injection the patient again experienced severe pain in spite of the im morphine sulfate. An additional 15 mg of morphine sulfate was necessary to ease the pain. The patient returned to the clinic in 1 month for his third course of chemotherapy. After premedication with 10 mg of iv morphine sulfate, vinblastine was given by iv drip in 150 ml of 5% dextrose solution infused over 45 minutes. The patient experienced a more bearable pain this time. He received three more courses of treatment in this manner, with recurrent pain. The patient had an excellent initial regression of his tumor and symptoms, but his disease later progressed.

Patient 2

Patient 2 was a 54-year-old white man with recurrent squamous cell carcinoma of the retromolar trigone treated originally with surgery and postoperative radiotherapy in October 1973. Recurrence was noted in December 1973, in the area of previous surgery and radiotherapy, and he was referred to us in April 1976. Combination chemotherapy was instituted as in patient 1. Due to the previous experience with other patients, vinblastine was infused over 45 minutes in 150 ml of 5% dextrose solution. Fifteen minutes after the start of infusion, the patient complained of severe pain at the site of tumor. The pain was similar in character and intensity to that described above. Meperidine (50 mg) was given im. The pain decreased over the following 2 hours. He returned to the clinic in 1 month and was given 8 mg of iv morphine sulfate before vinblastine infusion. The pain recurred. A second dose of 5 mg of iv morphine sulfate relieved his pain over the next 2–3 hours. Two mg of vincristine was given in place of vinblastine on his return visit to the clinic. No pain was noted by the patient. His disease slowly progressed over the next few months and he is currently receiving supportive care from his personal physician.

The pain syndrome noted in our patients bears similarity to what has been described by Stark and Fletcher. It appears to be uniform in onset, duration, and character. It occurs 1–3 minutes after iv injection of vinblastine and lasts from 20 minutes to occasionally 3 hours. The onset is abrupt. It is described by the patients as "severe stinging pain," "liquid fire," "bad toothache," and "burning." Pain occurs predominantly in the tissue where the tumor is detectable. In one instance, pain occurred in an area where presence of tumor was not obvious

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TABLE 1.—Relationship of response to vinblastine-associated pain

	No. of patients—		
	With pain	Without pain	Total
No. evaluable for response	9	8	17
No. with complete response* and partial response† > 50%	8‡	3‡	11
Partial response < 50% > 25%	1	4	5
No response	0	1	1

*Disappearance of all clinical disease for at least 2 mos.
†Reduction of measurable disease as noted above for at least 2 mos and appearance of no new areas of disease.
‡$P = 0.026$ (Student's t test).

until subsequent followup. The intensity of pain can be somewhat lessened by parenteral medication with meperidine or morphine sulfate. No obvious vasovagal or vasomotor disturbance has been noted. The purpose of our report is to show the reproducible characteristics of vinblastine-related pain and to suggest its correlation with the tumor localization and therapeutic response.

RESULTS

While evaluating the responses in our patients to date, a difference was noted between those who experienced vinblastine pain and those who did not (*see* table 1). Evaluable patients are defined as those who have received two or more treatments and have measurable disease. Because of the difference in response rates we feel that tumor pain on administration of vinblastine may be a positive indicator of tumor response. We will soon be reporting survival differences for this regimen.

DISCUSSION

The pain associated with vinblastine injection as described by Dyke is attributed to swelling of tissue resulting from cellular destruction and is usually delayed, occurring during the first or second day following treatment.[4] The syndrome seen in our series of patients and those of Stark and Fletcher differs from pain described above by Dyke in its onset and severity.

Stark and Fletcher proposed that this syndrome may be due to the vasoactive properties of alkaloids (eg, ergot, yohimbine, and strychnine). However, in two of our patients, when vinblastine was replaced by vincristine because of the unbearable pain, the syndrome was not experienced. The minor differences in the structural formulae of these two compounds seem to suggest that this explanation may not be sufficient (3). In all 12 patients who experienced this pain syndrome, it occurred only in the tumor-bearing area of the head and neck. In one patient, pain was experienced in an area where there was no clinically detectable tumor. One month later, tumor became obvious in that area which was outside the port of previous irradiation. Further observation is needed to see whether this agent may have value in the early detection of tumors similar to the use of bleomycin-indium[111] tumor scan in Hodgkin's disease.[5]

[4]Dyke RW. Personal communication, Mar 30, 1976.

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High Incidence of Cardiomyopathy in Children Treated With Adriamycin and DTIC in Combination Chemotherapy

Recent reviews have documented the low incidence (5%–10%) of cardiomyopathy associated with

[5]Jones SE, Lihan D, and O'Mara RE. Indium-bleomycin tumor scanning in hematologic malignancies. Presented at the meeting of the American Society of Hematology, Dec 1973.

TABLE 1.—Clinical and laboratory observations

Patient No.	Age (yrs)	Tumor	Radiation therapy (rads)	Vincristine or VM-26	Total adriamycin dose (mg/m²)	Total DTIC dose (mg/m²)	Maximum AST* (units/liter)	Cardiac failure	Results
1	8	Ewing's sarcoma, right 8th rib	4000 to chest	VM-26	420	8750	—	At 11 mos	Death at 28 mos; recurrent tumor and cardiac failure
2	14	Ewing's sarcoma, right 1st metacarpal	4500 to right hand	Vincristine	540	11,250	35	—	Continuous remission for 31 mos
3	6	Ewing's sarcoma, right ilium	4500 to right ilium	Vincristine	540	11,250	49	—	Continuous remission for 28 mos
4	11	Ewing's sarcoma, left femur	4500 to left femur	Vincristine	480	19,000	42	—	Death at 14 mos; recurrent tumor
5	6	Ewing's sarcoma, left ethnoid	3600 to upper face	Vincristine	480	10,000	54	At 8 mos	Death at 17 mos; cardiac failure and recurrent tumor
6	5	Ewing's sarcoma, left distal radius	None	VM-26	480	10,000	69	At 9 mos	Death at 10 mos; cardiac failure
7	4	Ewing's sarcoma, right ilium	4500 to right ilium	Vincristine	360	7500	188	At 10 mos	In remission with controlled cardiac failure at 11 mos
8	5	Ewing's sarcoma, right orbit	4500 to right orbit	Vincristine	420	8750	57	—	Remission at 11 mos
9	6	Fibrosarcoma of buttock	4500 to buttock	Vincristine	360	7500	—	—	Remission at 60 mos
10	7	Fibrosarcoma of neck	3500 to neck	VM-26	360	7500	46	—	Alive at 17 mos with recurrent tumor

*Normal range = 7–50 units/liter.

adriamycin doses of < 500 mg/m² except when mediastinal irradiation has also been used (1,2). We report an unexpectedly high incidence of cardiomyopathy in children treated with combination chemotherapy including adriamycin and DTIC.

Fourteen patients with small cell sarcomas (11 with Ewing's sarcoma, two with fibrosarcoma, and one with leiomyosarcoma) were treated with combination chemotherapy using adriamycin (60 mg/m² on Day 1) and DTIC (250 mg/m² on Days 1–5); they were also randomized to receive either vincristine (1.5 mg/m² on Day 1) or VM-26 (100 mg/m² on Day 1). The drug combination was given every 4 weeks for six courses and then every 6 weeks for 2 years or until relapse.

Four patients received a total adriamycin dose of ≤ 300 mg/m²; none of these four developed cardiac failure and they will not be considered further. The remaining ten patients received adriamycin doses of 300–540 mg/m² and details of their treatment are shown in table 1. Four of these patients developed drug-related cardiomyopathy. Of these, one had irradiation to the chest wall, two had irradiation to areas distant from the chest, and one had no irradiation. The serum aspartate aminotransferase (AST) was the highest value recorded before any one of the courses of combination chemotherapy. All patients who developed cardiomyopathy had an elevated serum AST on at least one occasion during treatment while only one of six who did not develop cardiomyopathy had an elevated serum AST. Patients 1, 5, and 6 had acute cardiac failure while patient 7 had mild symptoms of congestive cardiac failure. The cardiac failure in patients 1, 5, and 7 was successfully controlled, but two patients subsequently relapsed and died of recurrent tumor. Patient 6 had rapid progression of cardiac failure and died despite supportive measures with Digoxin. Autopsy on patients 1 and 6 demonstrated chronic cardiomyopathy with moderate interstitial fibrosis and pleomorphism of myocardial nuclei. There was no evidence of acute degenerative or inflammatory change.

The occurrence of cardiomyopathy in four of ten patients treated with standard doses of adriamycin is far higher than that reported by others (1,2) and higher than has been our previous experience with adriamycin or daunorubicin. Since two of the four children who developed cardiomyopathy were given vincristine, adriamycin, and DTIC while the other two received VM-26 instead of vincristine, it would seem unreasonable to implicate VM-26 in association with adriamycin as the causative agent. We have been unable to find any reports implicating DTIC as a cause of cardiomyopathy when used as a single agent, but it was the only common agent associated with adriamycin in the children in our study who developed cardiomyopathy. Despite the absence of reports of cardiomyopathy when DTIC and adriamycin were used in combination in the treatment of children with disseminated neuroblastoma (3) and disseminated sarcoma in adults (4), our findings raise the serious possibility that the simultaneous use of DTIC and adriamycin in the manner described in the protocol may increase the cardiotoxic potential of adriamycin. It is also possible that the high incidence of cardiomyopathy is a chance occurrence in the small number of patients treated. We think this is unlikely because in our previous experience with adriamycin we observed only the usual incidence of cardiomyopathy.

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Use of Methyl-CCNU and Procarbazine in Advanced Malignant Melanoma Resistant to DTIC Therapy

DTIC is the most commonly used agent for disseminated malignant melanoma. The response rates have been in the range of 20%–30% (1,2); thus, there is a large group of patients who fail to respond to DTIC. These patients have a poor prognosis and their median survival is usually from 3 to 5 months. They need other effective chemotherapeutic regimens after failure to respond to DTIC treatment. A combination of methyl-CCNU and

procarbazine was given as a "second attempt" chemotherapy to improve the result in this study.

Seventeen patients (12 men and five women) with histologically proven advanced malignant melanoma who previously had failed to respond to DTIC treatment were given methyl-CCNU (130 mg/m^2) every 6 weeks and procarbazine (70 mg/m^2) for 2 weeks every 6 weeks. All patients had measurable disease. One patient (6%) had a partial response, and in another patient (6%), the disease remained static. The duration of response in both patients was 3 months. The survival from start of therapy was 5 months in both these patients while the median survival of nonresponders was 3 months. Gastrointestinal toxicity in the form of nausea and vomiting was seen in seven patients (42%) and hematologic toxicity in three (18%).

When used singly as first agents, both drugs have shown some effect in previous reports (3,4); however, when used in combination after failure with DTIC therapy, the responses have been very poor. It appears that "second attempt" chemotherapy in advanced malignant melanoma results in poor response.

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ANNOUNCEMENTS

Postgraduate Program "Controversies in Applied Cancer Immunotherapy"

San Francisco, California, March 4, 1978

The University of California is presenting a 1-day seminar on "Controversies in Applied Cancer Immunotherapy" to be held at Children's Hospital, San Francisco, California, on March 4, 1978. *For further information please contact:* Extended Programs in Medical Education, University of California, Room 569-U, San Francisco, Calif 94143, or call (415) 666-4251.

Seminars in Oncology at Roswell Park Memorial Institute

Buffalo, New York

There will be a seminar on "Outside Agents in the Etiology of Cancer" on February 9, 1978, organized by the Department of Epidemiology, Roswell Park Memorial Institute. The chairperson is Dr. Josef Vana.

There is a $10.00 registration fee. *For further information contact:* Ms. Claudia Lee, Cancer Control, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

Fourth Annual Meeting of the Association of Community Cancer Centers

Washington, DC, January 27–29, 1978

The Association of Community Cancer Centers (ACCC) announces its Fourth Annual Meeting to be held Friday, January 27, to Sunday, January 29, 1978, at the Twin Bridges Marriott Hotel, Washington, DC. ACCC is an organization composed of physicians and other health care providers dedicated to improving the quality of cancer treatment and care at the community level.

The topics to be presented at the meeting will focus on the *Oncology Unit and its Components,* with emphasis given to development of the oncology unit, administrative planning and staffing, patient/family/staff education, psychosocial considerations for staff and patients, hospice and continuing care, and research and the oncology unit. The January 28th luncheon keynote speaker will be Dr. Arthur Upton, newly appointed director of the National Cancer Institute. This meeting is open to physicians, hospital administrators, nurses, rehabilitation specialists, and others interested in community cancer programs. Advance registration is recommended. Registration fees are $100.00 for ACCC members and $150.00 for nonmembers.

For further information please write: ACCC 1978 Conference, 6000 Executive Blvd, Suite 508, Rockville, Md 20852.

Third Annual "Symposium on Advances in Cancer Treatment Research"

Baltimore, Maryland, May 18–20, 1978

The Baltimore Cancer Research Center, the National Cancer Institute, and the Program of Continuing Education of the University of Maryland School of Medicine are pleased to announce their Third Annual "Symposium on Advances in Cancer Treatment Research" to be conducted in Baltimore, Maryland, May 18–20, 1978, at the Baltimore Hilton Hotel.

An experienced and internationally recognized faculty will participate. This symposium is designed to update physicians and surgeons in some of the most recent developments in cancer treatment and current research including areas of clinical management, etiology, and cellular biology. Faculty members will be available throughout the symposium to discuss any questions or interests of individual course registrants. A visit to the Baltimore Cancer

Research Center can be scheduled for interested persons. This course is accredited for 16 hours of Category 1 credit for the PRA of the AMA by the Program of Continuing Education, University of Maryland School of Medicine.

Heinz Karger Memorial Foundation Award

In memory of the publisher Heinz Karger, an international prize for an important paper on a particular scientific subject is awarded each year. The Council of the Memorial Foundation includes Prof. Dr. J. Girard (Basel), Prof. Dr. G. Mayor (Zürich), Prof. Dr. J. R. Rüttner (Zürich), Prof. Dr. H. Studer (Bern), and the publisher Dr. h. c. Thomas Karger.

The Foundation invites the submission of original research papers to compete for the 1978 award on the following subject: Cytological and Histochemical Approach to the Diagnosis of Tumors. Manuscripts marked *Competition* must reach the publishers, S. Karger AG, Arnold-Böcklin-Strasse 25, 4011 Basel (Switzerland), not later than February 28, 1978. To be eligible for consideration, a manuscript must not exceed 20 typewritten pages, including illustrations, tables, and bibliography. It should be typewritten on one side only, double-spaced, and submitted in quadruplicate, in accordance with the instructions contained in the *Rules for the Preparation of Manuscripts* (this booklet can be obtained free of charge from the publishers if the request is marked *Competition*). The language of the paper should be English, German, or French. The winning papers will be published in English in one of the Karger journals.

The award will be SFr. 7000. The Council of the Foundation will judge the papers and confer the prize.

Medical Oncology Fellowships

Applications are now being accepted for fellowships in medical oncology at the Los Angeles County-University of Southern California Medical Center to begin July 1978 and July 1979. The program will be under the direction of Malcolm S. Mitchell, MD. Applicants should be board-eligible in internal medicine. The salary is $21,252 for the first year. *Address inquiries to:* John E. Bethune, MD, Professor and Chairman, Department of Medicine, Rm 7900, 1200 N. State St, Los Angeles, Calif 90033.

Symposium on "Designs for Clinical Cancer Research"

Monteleone Hotel, New Orleans, Louisiana, April 13–15, 1978

The Division of Cancer Treatment and the Clinical Investigation Review Committee of the National Cancer Institute (Bethesda, Md) will sponsor a symposium on "Designs for Clinical Cancer Research" to be held April 13–15, 1978, at the Monteleone Hotel in New Orleans, La.

The purpose of the symposium is to explore the statistical principles and clinical realities for various experimental designs. Major topics to be discussed jointly by physicians and statisticians include: "Basic Design Considerations," "Adequate and Appropriate Controls," "Selection, Use, and Verification of Prognostic Factors," "Critical Definitions and Criteria for Evaluation," "Optimal Combinations for Modalities," and "Special Problems and Unusual Parameters." The Program Chairman is Dr. Nell Sedransk and the Program Co-Chairman is Dr. Stephen K. Carter.

Sessions are open to all members of the medical- and health-related professions. Advanced registration is requested. There are no registration fees. *For information write:* Mr. C. W. White, Executive Secretary, Cancer Clinical Investigation Review Committee, Division of Cancer Treatment, Landow Bldg, Rm C809, National Cancer Institute, Bethesda, Md 20014.

13th Annual San Francisco Cancer Symposium on "Combined Effects of Chemotherapy and Radiotherapy on Normal Tissue Tolerance"

Hyatt Regency Hotel, San Francisco, California, March 3–4, 1977

The West Coast Cancer Foundation announces the 13th Annual San Francisco Cancer Symposium on "Combined Effects of Chemotherapy and Radiotherapy on Normal Tissue Tolerance" to be held March 3–4, 1977. The meeting has been approved for CMA Category I credit.

Registration is $100.00 prior to February 17, 1977, and $125.00 after February 17, 1977, or at the door. *For further information contact:* J. M. Vaeth, MD, or Judith L. Kohn, West Coast Cancer Foundation, 50 Francisco St, Suite 200, San Francisco, Calif 94133 (telephone 415-981-4590).

Staff Positions and Fellowship Training: Hematology/Oncology

The US Army Medical Corps has several staff positions available for clinicians who are board certified/eligible in hematology and/or medical oncology. Potential assignments are available in the continental US and in Europe at a number of medical centers and larger station hospitals. Fellowship training is available to US citizens who are board certified/eligible in internal medicine at Brooke Army Medical Center (San Antonio, Tex), Fitzsimons Army Medical Center (Denver, Colo), Letterman Army Medical Center (San Francisco, Calif), and Walter Reed Army Medical Center (Washington, DC).

Inquiries should be addressed to: Daniel B. Kimball, Jr, MD, Consultant to the Surgeon General for Hematology/Medical Oncology, OSC Box 336, Walter Reed Army Medical Center, Washington, DC 20012 (or call 202-576-3260).

Chemotherapy Fellowships

The Brain Tumor Research Center has openings beginning July 1, 1978, to train neurosurgeons, neurologists, and clinical oncologists in clinical neuro-oncology and laboratory research. One or 2 years may be elected, and stipends are variable depending on previous training.

Send a resumé and three letters of reference *before April 1, 1978,* to: Victor Levin, MD, Co-Director, Chemotherapy Service, Brain Tumor Research Center, Department of Neurosurgery, University of California, San Francisco, Calif 94143 (An Equal Opportunity/Affirmative Action Employer).

Symposium on "Controversies in Cancer Treatment"

Brussels, Belgium, April 26–29, 1978

The European Organization for Research on Treatment of Cancer (EORTC) is sponsoring a symposium on "Controversies in Cancer Treatment" to be held in Brussels, Belgium, April 26–29, 1978 (2 rue Evers, 1000 Brussels [1st floor]). The first day of this symposium will be devoted to controversies on design and analysis of clinical trials.

Attendance will be limited to the first 200 registrations; the registration fee is 3000 Belgian francs. Abstracts (in English) of free communications on cancer therapy must be submitted *by February 15, 1978* (to be presented on April 29). *For details and registration, write to:* Dr. M. Staquet, EORTC Data Center, Institut Jules Bordet, rue Héger-Bordet 1, 1000 Brussels, Belgium.

CORRECTIONS

An error appears on page 1561 of the November 1977 issue in the article "Observations on the Human Pharmacology of Cycloleucine." The first line of the second column should read "chloroform *as an* internal standard."

The legend for figure 23 (page 1552) of the article "Whole-Body Computed Tomography in the Diagnosis of Abdominal and Thoracic Malignancy: Review of 600 Cases" in the November 1977 issue was accidentally omitted. The legend which should be inserted is as follows:

FIGURE 23.—23-yr-old woman with symptoms of retroperitoneal bleeding following percutaneous renal biopsy. (A) CT scan defines a perirenal hematoma (→) lying lateral and posterior to the left kidney. (B) 30 days later the hematoma has undergone nearly complete resolution.

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